CHEMICAL STERILIZATION OF THE STABLE FLY, *Stomoxys calcitrans* (LINNE), WITH METEPA AND HEMPA

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INTRODUCTION

The stable fly, Stomoxys calcitrans (L.), also known variously as the dog fly, beach fly, or biting house fly, is widely recognized as an important insect pest. It causes economic loss in the livestock industry and is an insufferable nuisance to man in rural and resort areas.

Cheng (1958) found that in cattle the mean gain in weight per animal per day was 1/2 to 2/3 lb greater in animals protected from biting flies, the stable fly included, than in the control animals. Cutkomp and Harvey (1958) were able to accomplish 95% control of horn flies (Haematobia irritans) and 70% control of stable flies on cattle, and thereby obtained an average daily gain in weight of 1.3 lb per animal as against 0.63 lb in the untreated checks.

Bruce and Decker (1957, 1958) found a significant correlation between stable fly abundance and reduction in milk and butterfat production in dairy cattle. The average monthly rate of loss was 0.65 to 0.7% per fly per cow. The depressed production would continue for weeks and months beyond the end of the fly season.

Melvin (1932) has reported some physiological effects of the stable fly on cattle. Under conditions favorable for feeding activity, 100 flies caused no noticeable rise in body temperature; 200 flies per cow caused a rise of about 0.2 to 0.6 F, and 300 flies per cow caused a rise of 0.4 to 1.0 F. Six thousand flies feeding on a young heifer (1 1/2 years old) caused a rise of 6.4 F in just one hour. House flies did not cause any rise in body temperature, even when molasses was sprayed on the cows.

Hansens (1951) has reported the stable fly as an important pest in resort areas on the New Jersey coast. It may be the cause of severe economic losses, as a few hundred flies are sufficient to drive bathers off the beaches. The stable fly is also a serious pest along the beaches in Florida. According to Blakeslee (1945), it breeds along the coast of the Gulf of Mexico in northwest Florida, from Pensacola to about the St. Mark's river, for a distance of over 200 miles and is a perennial major pest during late summer and early fall.

The stable fly generally is not considered important as a vector of animal or human diseases. However, Horsfall (1962) states that because the flies tend to probe the skin of one or more animals in their feeding, they can serve as carriers of contaminants.

According to Herms (1961), the stable fly is somewhat important in the mechanical transmission of infectious anemia of horses (a virus), of the anthrax bacillus, and of trypanosomiasis, especially Surra of horses, mules and camels, caused by Trypanosoma evansi (Steal). Richard and Pior (1966) have shown experimentally that the fly can act as a mechanical agent in the transmission of cutaneous streptothricosis from infected rabbits to healthy ones. This skin disease, caused by Dermatophilus congolensis also attacks cattle, horses, goats, game species, and man.

Given the importance of the stable fly, it is obviously in man's best interest to combat it by all available means. At present such means include cultural methods, use of insecticides at the breeding places, and insecticide-repellent treatments for the protection of livestock.

A good measure of control can be achieved by the proper disposition of vegetable waste at harvest time. According to Horsfall (1962), the advent of the combine resulted in the dramatic decline of populations

of stable flies in the plains and prairies of the central United States. Formerly straw piles were a feature of the landscape, but now the straw is scattered, and thus a major breeding source of stable flies has been eliminated in those regions.

Simmons and Dove (1942a) found that waste celery left around the processing plant, as well as piles of peanut litter left in the fields, serve as an excellent breeding medium for the stable fly. However, if the wastes were scattered in the fields they were not important as a breeding medium.

Bishopp (1939) advises the proper disposal of all sorts of animal refuse. Bedding soiled with manure should be scattered thinly to allow it to dry, and manure piles should be properly screened and preferably equipped with suitable fly traps.

The use of insecticides for control at the naturally occurring breeding grounds has also been recommended. Simmons and Dove (1942, 1945) were able to control the stable fly in drifts of marine grasses by spraying them with creosote (10%), mixed in bay water, or by the use of "gas condensate". Blakeslee (1945) used DDT (0.5%) or DDT residual oil (2.5%) in bay water and obtained 90-95% mortality of emerging adults. Hansens (1951) obtained similar results with DDT, methoxychlor or TDE, all at 0.5% in bay water.

Most efforts, however, have been aimed at controlling the fly in the barn. The goal has been to protect livestock by means of insecticide-repellent formulations applied to resting surfaces and to the animals themselves. At best the use of these formulations on cattle achieves only a short-lived inefficient protection against the attack of biting flies and repeated applications are necessary.

The use of systemic insecticides to protect cattle has also been extensively explored. In a special report on the screening of animal systemic insecticides, Drummond (1961) reported 49 compounds to be effective against the stable fly.

The treatment of cattle with insecticides, both residual and systemic, may result in the presence of pesticide residues in the meat and milk (Claborn et al., 1960); and the application of insecticides to the naturally occurring breeding areas may contribute to the undesirable contamination of the environment. An up to date review on the consequences of the use of insecticides, as it affects non-target organisms is given by Newsom (1967).

The most recent recommendations for the protection of cattle by means of insecticides are contained in the U.S.D.A. Handbook 313, published in 1966.

A goal more ambitious and worthwhile than merely protecting cattle would be the eradication of the stable fly. This goal might be attained by the male sterility method, either by the rearing and subsequent release of large numbers of sterile males, or by sterilizing the natural population without widespread contamination of the environment.

As reported by Christenson (1966), the sterile insect release method, originally conceived by Knipling about 1937, has proved its effectiveness against populations of the screw-worm fly, Cochliomyia hominivorax (Coquerel), the melon fly, Dacus cucurbitae (Coquillett), the oriental fruit fly, Dacus dorsalis (Hendel), the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), and the Mexican fruit fly, Anastrepha ludens (Loew.).

Knipling (1964) has enunciated 9 basic requirements and factors which determine the feasibility of the sterile insect release technique. These are:

a. Availability of a method of inducing sterility without serious adverse effects on mating behavior and competitiveness.

b. Method of rearing the insect.

c. Quantitative information on natural population density at the low level in the population cycle.

d. A practical way of reducing natural populations to levels manageable with sterile insects.

e. Information on rate of population increases as a guide for determining the necessary rate of overflooding with sterile insects.

f. Cost of current methods of control plus losses caused by the insect must be higher than the cost of reducing the natural population plus the cost for rearing and releasing the required number of sterile insects.

g. If complete population control cannot be maintained because of reinfestations by migrating insects, or new introductions, the cost of maintaining complete control by continuing sterile-insect releases must be favorable in relation to the costs for current methods of control, plus additional losses caused by the insects.

h. There would be justification for employing the sterile-insect-release method, even if it were more costly than current ways to control or eradicate insect populations, if it provides advantages in overcoming hazards to man and his environment.

i. Sterile insects to be released must not cause undue losses to crops or livestock, or create hazards for man that outweigh the benefits of achieving or maintaining population control.

The stable fly is an insect pest that might be properly controlled by the sterility method. It can be mass reared, it has good powers of dispersal, and it is an important pest which causes significant economic losses, and demands a continued and costly control program. Jackson (1966) believes that the stable fly is an obvious candidate for the sterility method of control.

The work herein reported was undertaken in connection with the first requirement listed above. The specific objectives were:

1) To determine the range of effectiveness of metepa (tris(2-methyl-1-aziridinyl)phosphine oxide) and hempa (tris(dimethylamino)phosphine oxide) as chemosterilants of the stable fly;

2) To determine possible effects of sterilizing doses on the longevity of the stable fly;

3) To determine the toxicity of both compounds on the stable fly and estimate the safety factor for their practical use; and

4) To determine possible effects of sterilizing doses on the mating competitiveness of the stable fly.

REVIEW OF LITERATURE

Biology of the Stable Fly

The egg

The egg of the stable fly has been described by several authors (Newstead et al., 1907; Parr, 1962). It is pale white when newly laid but changes to creamy white before hatching. It is elongated, slightly curved, and measures about 1 mm in length by 0.2 mm wide. On the concave side of the egg is a deep longitudinal groove, which bifurcates at the anterior end to form an operculum. The chorion is thick and coriaceous bearing faint reticulations. The egg shells do not collapse or shrivel after hatching.

Eggs of the stable fly are usually laid in a moist medium, which is very favorable for hatching. Under conditions of stress, such as caged females not provided with oviposition medium, the eggs may be laid on a dry surface, such as the gauze of the cage or the surface of the feeding tube. Eggs laid on a dry medium at an atmospheric humidity of 55-65%, or lower, desiccate rapidly and fail to hatch.

The time required for the eggs to hatch varies mainly with the temperature. Newstead et al. (1907) found that the incubation period of the stable fly eggs was 8 days at a temperature varying from 18 to 19.5 C. Melvin (1931) determined that the mean incubation period was 33.4 hours at 25 C and 26.5 hours at 30 C. According to Champlain et al. (1954) there is no hatching at 12 C and the eggs gradually lose viability. He was able to store eggs immersed in water, and obtained only 50% hatching after one week of storage. Jones (1966) has stored stable fly eggs

under moist conditions or in water at 7 C and has obtained 50 to 75% hatching after one week of storage. He reported the incubation period thus: two days at 15.5 C, one day at 21.1 C, and one day at 26.6 C.

In the act of hatching the young larva forces open the operculum and emerges rapidly. The hatching process takes about 14 seconds (Parr, 1962).

The larva

The larval stage of the stable fly comprises three instars. Tao (1927) has described the cephalopharyngeal characteristics of the first and second instars to distinguish them from other common flies. Newstead et al. (1907), Patton and Evans (1929), Greene (1956), and Parr (1962) have described the third instar or fully grown larva of the stable fly. The larva is a typical muscid maggot; milky white in color, cyclindrical in shape, tapered toward the anterior end, and composed of eleven segments plus the head, the last segment being widely rounded. The ventral surface of the last seven segments is furnished with raised bands bearing fine, black spines used as ambulatory organs. The anterior spiracles have five finger-like processes. The posterior spiracular plates are black, triangularly shaped with rounded apices, and are well separated; the circular button is located at the center of each plate, and the slits appear yellowish and resemble the letter S. Each slit is located in a pale whitish area.

The newly emerged larva measures an average of 1.08 mm in length, and grows to 1.7 mm, the second instar attains a length of 2.80 mm, and the third instar 11.12 mm when fully grown (Parr, 1962).

Length of the larval period.-- The duration of the larval stage is dependent upon the temperature and probably the nutritive value of the medium. Melvin (1931) reported the length of the total larval period thus: 15.5-15.7 days at 25 C and 13 to 13.6 days at 30 C. Melvin reared larvae in a mixture of equal parts of alfalfa meal and wheat bran. Jones (1966), on the other hand, reported considerably shorter development times in CSMA standard fly larval medium, i.e., 18 days at 15.5 C, 13 days at 21.1 C and 7 days at 26.6 C room temperatures. Under the rearing conditions at the U.S.D.A. laboratory in Gainesville, Florida, the length of the larval stage was 8 to 9 days at 25.5 C.

Parr (1962) recorded the average length of the larval period by instars in a rearing room maintained at 26.6 C and 80% relative humidity. He found first instar larvae after the first 24 hours, second instar larvae after 48 hours and third instar larvae from the 3rd to 8th day.

In nature, the duration of the larval stage of the stable fly varies considerably. According to Bishopp (1939) the larval growth is completed within 11 to 30 days. Simmons and Dove (1942a) reported 11 to 15 days for the larval development in celery stripping in the month of May in Florida. Hansens (1951) did not find any larvae of Stomoxys when the temperature of decaying matter was above 31.1 C; heaviest breeding occurred between 20 and 25.5 C. In nature, the 3rd larval instar may endure for months and serves as the overwintering stage (Simmons and Dove, 1942a).

Natural breeding medium of the larva.-- In nature, the larvae of the stable fly breed in horse manure, soiled stable breeding, waste feed and silage, and, perhaps more importantly, in fermenting vegetable matter.

Newstead et al. (1907) found larvae of the stable fly breeding in lawn clippings under moist conditions. Bishopp (1913) refers to an exceedingly severe outbreak of stable fly in 1912. It covered northern Texas, Oklahoma, and the entire grain belt of the U.S. It was determined that the majority of the flies bred in straw stacks, more abundantly in oat straw than in wheat straw. Alfalfa stacks were generally uninfested. As previously mentioned the use of the combine harvester did away with this source of flies by scattering the straw (Horsfall, 1962).

In northwestern Florida, Simmons and Dove (1941, 1942) investigated the sources of stable fly outbreaks that occurred annually in the spring, late summer and early fall, and late fall. They found that the summer outbreaks were the result of heavy breeding in fermenting deposits of bay grasses washed ashore, mainly Shoalgrass, Halodule wrightii, Aschers, and Turtlegrass, Thalassia testudinum Koenig and Sims. The late fall outbreaks originated from breeding in piles of peanut litter left in the fields after harvest. The spring outbreaks originated from piles of waste celery that accumulated near processing plants. If the waste celery was plowed under after being infested the flies would still emerge.

Parr (1962), working in Uganda, where long, dry periods occur and high temperatures predominate, found that the stable fly breeds mostly in the rotted cattle manure mixed with rotted straw, grass or leaves, which is found in the wet, shaded portions of cattle "bomas" or corrals. Herms (1961) found it breeding in decayed onions in the autumn.

All observations on stable fly breeding indicate that porosity is an important characteristic of the medium. No stable fly breeding has been found in fresh, compact cattle manure or in human excrement. In

the laboratory, the standard CSMA fly larval medium alone is not adequate for rearing the stable fly, but the addition of a coarse material, such as wood shavings, vermiculite, oat hulls, etc., makes the medium satisfactory. Jones (1966) has summarized what is known about laboratory media for rearing the stable fly.

Habits of the larvae.-- The larvae of the stable fly begin feeding as soon as they hatch from the eggs and seem to feed continuously up to the prepupal period. Parr (1962) did not detect any resting period prior to ecdysis and concluded that molting in no way interfered with larval feeding and growth.

The larvae feed concealed inside the medium at all times and immediately crawl deeper when uncovered. This observation led Newstead et al. (1907) to conclude that the stable fly larvae require complete darkness for satisfactory development. Thus, Parr (1959) deemed it necessary to rear his larvae in a dark room. Other workers, however, have not confirmed the requirement for darkness. Jones (1966) has successfully reared the stable fly under conditions simulating a 16 hour photoperiod, and further recommends that the medium be stirred daily, thus uncovering the larvae. The colony used for the work herein reported was maintained under continuous lighting without any apparent detrimental effects. It was possible to see the developing larvae moving about and feeding nearly in contact with the transparent glass of the rearing jar.

When fully grown, the larvae stop feeding and migrate to drier portions of the medium, usually near the surface, where they enter a period of quiescence and finally pupate. Pupation may occur at any point on the surface of the medium, but more commonly the full grown larvae congregate and pupate around the periphery. If the medium

is too wet, the larvae have been observed to crawl up the walls of the rearing jar and pupate under the cloth cover.

The pupa

Pupal development of the stable fly takes place inside a puparium, as is typical of all the muscid flies. Detailed accounts on the formation of the puparium have been given by Newstead et al. (1907), Mitzmain (1913) and Parr (1962). The puparium consists of the hardened cuticle of the fully grown larva. "Pupal characters" are those of the puparium.

The puparium is orange colored at first, turning to reddish brown in 3 days and to a dark, nearly black color prior to eclosion of the adult. Newstead et al (1907) describe the puparium as being barrel shaped, slightly narrowed in front and broadly rounded behind. Two large disc shaped spiracles are present at the posterior end. Eleven segments are visible. Parr (1962) measured 100 puparia and reported the following mean values: length 5.27 mm; width 1.96 mm and weight 11.23 mg (range 8.00 to 14.00 mg).

The duration of the pupal stage is variable, principally dependent upon temperature. According to Jones (1966), pupae of the stable fly cannot be stored satisfactorily, as only a few air-dried pupae will remain alive at 5 to 8 C for a maximum of 14 days, while at 10 C emergence can occur. Jones also gives the following figures for the pupal stage: at 15.5 C, eight days, at 21.1 C, seven days and at 26.6 C, seven days. Melvin (1931) reported a pupal stage of 7.4 days at 25 C and 100% relative humidity. The stable fly pupae reared in the U.S.D.A. laboratory in Gainesville required 7 days for eclosion at 25.5 C and 60% relative humidity.

Relative humidity may influence pupal development of the stable fly. Mitzmain (1913) found that pupae immersed in water for 7 days failed

to develop. Melvin (1931) obtained no emergence from pupae kept at 19% and 0% relative humidity and 25 C; 99.8% emergence was obtained, however, from pupae kept at 100% relative humidity. In nature, the pupal stage lasts from 5 to 6 days under favorable conditions (Mitzmain, 1913) and up to about 3 weeks in cool weather (Bishopp, 1939).

The adult

The adult stable fly greatly resembles the house fly (Musca domestica L.). Nevertheless, it can be readily distinguished from the latter. It has piercing sucking mouth parts with a proboscis pointing forward from under the head. The abdomen is larger and more squarely shaped than that of the house fly, and, on the dorsal side, it bears seven rounded dark brown spots arranged in a characteristic checker-like pattern.

The stable fly is classified by Patton and Evans (1929) as follows:

Class: Hexapoda

Order: Diptera

Suborder: Cyclorrhapha

Family: Muscidae Calypterata

Subfamily: Muscinae

Genus: Stomoxys

species: Stomoxys calcitrans

The same authors characterize the genus Stomoxys as follows:

> Genus Stomoxys. CHARACTERS Antenna with simple spinulae only on upper surface of arista. Proboscis strongly chitinized and projecting forwards, and tapering slightly towards extremity; bulb of proboscis well developed; palps very small and not seen when proboscis at rest. Mesonotum longer than broad, and usually marked either with two, or four clove brown stripes. Wing venation characteristic; fourth long vein, $M_1 + 2$ bends forwards towards the third long vein, R_{4+5} with a gentle curve ending at margin of wing some distance behind third vein, R_{4+5}; first posterior cell, R_5 widely open. Abdomen either

with round, clove brown spots, or with only bands. Both ♂ and ♀ are blood-suckers. Many species, S. calcitrans the commonest, and most widely distributed.

The stable fly was originally named Conops calcitrans by Linnaeus in 1758. Later, in 1762, the genus Stomoxys was created by Geoffroy, and the stable fly was transferred to said genus. The following account of Stomoxys calcitrans (L.) is given by Huckett (1965).

Genus Stomoxys Geoffroy

Stomoxys Geoffroy, Hist. Ins., 2: 538, 1762; Townsend, Ann. ent. Soc. Amer. 7: 160-167, 1914; Malloch, Ann. Mag. nat. Hist. (10)9; 381, 1932.
Type species: Conops calcitrans Linnaeus

Stomoxys calcitrans (Linnaeus)

Conops calcitrans Linnaeus, Syst. Nat., p. 604, 1758.
Evpis calcitrans Scopoli, Ent. carniol., p. 368, 1763.
Stomoxys calcitrans Fabricius, Syst. Ent., p. 798, 1775.
Stomoxys parasita Fabricius, Ent. Syst., 4: 394, 1794.
Stomoxys inimica Robineau-Desvoidy, Essai Myod., p. 387, 1830.
Stomoxys cybira Walker, List. dipt. Ins. Brit. Mus., 4: 1159, 1849.
Type locality: Europe

Emergence.-- The emergence of the adult stable fly has been noted in detail by Newstead et al (1907). The newly emerged fly is pale gray in color and the body is greatly elongated. Its wings are crumpled and its mouth parts are appressed to the ventral side between the legs. Upon eclosion, the fly walks rapidly to a suitable resting place and there the proboscis folds forward and attains its characteristic position. Within a half hour the body darkens, the wings expand and the insect is ready to fly away.

Feeding.-- The adult stable fly is a true blood feeder. Both the male and female suck the blood of animals. No record was found stating that it could reproduce on any other food, although it feeds readily on other fluids both in nature and in the laboratory (Bishopp, 1913). Herms (1961) found that stable flies which fed only on sugar water deposited

no eggs, although many survived 20 days or longer. Downes (1958) has reviewed the literature on the feeding habits of biting flies; and concludes that Stomoxys calcitrans (L.), among others, requires sugar to sustain life and protein for ovarian development. According to Lotmar (1949), sugar solutions go to the crop but blood goes directly to the midgut; and furthermore, the hunger reaction of Stomoxys is not satisfied by a crop full of sugar solution so that a blood meal can still be taken. Day (1954) has suggested that this arrangement would be valuable for survival when hosts are found only occasionally.

Tuttle (1961) studied the nutritional requirements of the stable fly and found that whole beef blood could even be diluted (2 parts blood to 1 part tap water or saline solution) and fed to stable flies without impairment of egg production or longevity. Higher dilutions were not adequate for egg production and resulted in high premature mortality. At least 6 blood meals in 9 days were necessary for good oviposition, and neither the serum nor the red-cell elements fed separately to stable flies induced good oviposition. Beef blood serum supplemented with dextrose 0.25 or 0.50 molar sustained life but resulted in delayed egg production. The number of eggs, however, was comparable to that of the controls. Beef blood reconstituted from dried blood and saline (2:8) did not sustain life long enough for oviposition. When the stable flies were left without food for 48 hours or more high mortality resulted.

The stable fly can feed on the blood of many species of animals. Mitzmain (1913) showed that the stable fly could feed on 17 species of animals in as many days. Man, most domestic animals, including chickens, and even a bat and a lizard were included in his experiment. Mitzmain concluded that the stable fly could feed on any animal which submitted

to its attacks. In nature, however, the stable fly probably feeds mostly on large animals, such as cattle, horses, hogs, sheep, goats and the like. The stable fly has been observed to feed mostly at dawn and in the late afternoon under natural conditions but it can feed at any time during the daylight hours (Mitzmain, 1913).

The average amount of blood taken by a hungry stable fly, i.e. unfed for 24 hours, was estimated by Parr (1962) at 25.8 mg, or about three times its body weight. Hopkins (1964), on the other hand, found that the mean volume of blood consumed by flies allowed to engorge was only from 8 to 12 microliters.

The manner of feeding of the stable fly has been observed and described by Newstead et al. (1907) and by Mitzmain (1913). The whole proboscis acts as a piercing organ. The labella are provided with toothlike sclerites which rupture the skin by means of rotary movements, and thus open the way for deep penetration of the proboscis, up to half its length into the skin of the host.

In this respect, the mouth parts of the stable fly are worthy of special note. They represent a modification of the generalized piercing-sucking type of mouth parts. The piercing apparatus consists of the labium, the hypopharynx and the labrum-epipharynx. The anatomy of the mouth parts of the stable fly was studied and described in detail by Stephens and Newstead (1907) and Brain (1912, 1913).

Mating.-- The mating habits of the stable fly have only recently been studied in the laboratory. Killough and McKinstry (1965) worked under conditions simulating a 16 hour photoperiod, at 25.5 C to 28.3 C and 46 to 53% relative humidity. When caged together at a ratio of 3 ♂ to 1 ♀ , one day old males successfully fertilized 13% of five day old

females in a 24 hour period. Five day old males, however, fertilized 53% of the females in a 24 hour period. When five day old males were caged with one day old females at a ratio of 1 ♂ to 3 ♀ they found that 6% of the females were successfully fertilized within one day.

Harris et al. (1966) conducted experiments with males and females of the same age, under continuous lighting and at 26.6 C and 50-60% relative humidity. They found that only 1% of the females had mated between the first and second day after emergence. When the flies were 5 days old, 89% of the females had mated. When newly emerged males were caged with 5 day old virgin females at a ratio of 1 ♂ to 2 ♀ , no males mated on the first day, but 24% had mated by the second day and 95% had mated by the fifth day.

Harris et al. (1966) also showed that a single male was able to inseminate an average of 6.3 females and as many as 9 females. Females were shown to mate only once if successfully inseminated, but would mate a second time if not successfully inseminated. Only about 60% of the females mated successfully the first time. Mating time varied from 3 to 7 minutes. The mating habits of the stable fly in nature apparently have not been studied as no pertinent record was found in the literature.

Oviposition.-- In nature, the stable fly lays its eggs on moist, fermenting vegetable medium. Several such media are mentioned previously. Moisture and the vapors given off by suitable fermenting vegetable matter seem to afford some stimulus for oviposition.

Newstead et al. (1907) noticed, after disturbing a heap of fermenting grass mowings, that female stable flies appeared in a matter of minutes and oviposited down to a depth of 7.5 cm in the moist medium. Bishopp (1913) also observed female stable flies darting into straw stacks to lay eggs as soon as he removed the dry surface of the stacks.

In the laboratory, caged stable flies readily lay eggs on cloth or cotton pads well moistened with water or with a 1-5% solution of ammonium hydroxide (Jones, 1966). The spent larval rearing medium gives off a strong odor of ammonia, and is a most suitable oviposition medium for the stable fly in the laboratory. Eggs are also laid abundantly on the blood-soaked cotton pads used for feeding the caged stable flies. As pointed out earlier, if no suitable oviposition medium is provided to caged stable flies, they will lay eggs on the dry screen and bottom of the cage.

The stable fly may lay its eggs singly (Patton and Evans, 1929) or, most commonly, in small egg masses. Newstead et al. (1907) recorded 7 egg batches ranging from 48 to 71 eggs per batch, and in some instances they saw the female separate the eggs with her proboscis and then drag and scatter them with her legs.

Under laboratory conditions, Parr (1962) was able to collect either 10 or 11 egg batches from each of 5 females, for a total of about 375 eggs per female. The average number of eggs per batch was 35.5, but one batch contained 96 eggs. Mitzmain (1913) obtained a maximum of 632 eggs from a female that lived 65 days. Killough and McKinstry (1965) collected batches containing 40 to 80 eggs with a range of 1 to 184 eggs per batch. One individual laid a maximum of 602 eggs.

According to Mitzmain (1913), the preoviposition period of the stable fly in the laboratory is 9 days; Parr (1962) obtained eggs on the 8th day, Hopkins (1964) first noted eggs on the 6th day, and according to Killough and McKinstry (1965) the female stable fly will not lay eggs before she is 8 days old. Jones (1966) noted a temperature effect and recorded the following preoviposition periods: 18 days at 15.5 C; 9 days

at 21.1 C and 6 days at 26.6 C. Killough and McKinstry (1965) also found that virgin females not exposed to other flies (caged alone) did not lay any eggs. However, the author has obtained good numbers of eggs, all sterile, from 15 day old virgin females caged in groups of 25.

Habitat.-- It is generally accepted that the stable fly occurs wherever there is livestock, whether in the open range or in shelters. It is frequently found in stables, resting on walls, beams and rafters (Bishopp, 1913) or upon trees and fences bordering corrals and sheds (Mitzmain, 1913). Somme (1958) noted that of 5,954 flies observed in 11 barns in Norway during the summers of 1956 and 1957, stable flies comprised 74.5%, house flies accounted for 15.3% and Fannia comprised the remaining 10.2% of the fly population.

Dispersal.-- Although there is very little information regarding the dispersal of this fly, it apparently ranges widely over the countryside. Bishopp and Laake (1921) mentioned that Hodge observed house flies, stable flies and blue bottle flies at cribs 5 and 6 miles out in Lake Erie and believed that they had been blown out there. Simmons and Dove (1942) reported that localized outbreaks of the stable fly extended 8 to 12 miles from the waste celery deposits where it was breeding. Hansens (1951) observed in resort areas in New Jersey that the fly would appear with the winds from the west and would disappear as rapidly with a change in wind direction.

Eddy et al. (1962) released several thousand P^{32} and fluorescent-marked stable flies, horn flies (Haematobia irritans (L.)), house flies, and mosquitoes (Culex tarsalis Coquillet, C. peus Speiser, and Aedes dorsalis (Meigen)). The stable flies showed the most rapid dispersion,

with specimens being recovered 5 miles from the release point in less than 2 hours. Flight movement was favored by wind direction.

Response to stimuli.-- The literature contains a few reports on the reaction of the stable fly to stimuli, mainly in regards to orientation, feeding stimuli, attraction, and repellency. Dahm and Raun (1955) observed that stable flies apparently were not attracted to baits, and noted also that these flies seemed to prefer to rest on vertical surfaces, so that the (Scudder, 1947) fly grill was not adequate to measure populations of this insect. According to Hansens (1951), the stable fly is attracted to dark colors: He counted 20 times as many flies on dark blue trousers as on light blue ones in a period of 10 minutes. He also noted that the flies attacked people usually from the knee down.

According to Parr (1962), a black cloth screen carried through the bush attracted the stable flies and provided a good method for capturing them in the field. Also black cows were invariably more heavily attacked than light colored cows. He felt, however, that color and smell operate only at close proximity, while at long range the movement of the host was the important factor. Flies were attracted to a moving screen but not to a stationary one.

Gouck and Gilbert (1962) made observations on the responses of mosquitoes and stable flies to a man wearing a light-weight rubber diving suit which completely covered him and prevented the release of water vapor, carbon dioxide, or other gaseous or volatile substances into the surrounding environment. When the subject wore the diving suit with no white oversuit the total number of landings of the stable fly was usually no greater, and frequently less, than on a dummy, similarly dressed, except when the face was exposed. With a white oversuit the subject received about the same number of landings as the dummy.

In the same series of experiments damp suits and exposed hands did not increase the landing rate of the stable fly, but significantly more landings per 20 sec. were received on a soiled suit (12.7), on clean suits with face (10.7) or hands and face (9.1) exposed, and on a white suit with no diving suit (11.2). In these tests no carbon dioxide was discharged over the head of the subject. In the tests in which carbon dioxide was used the counts on the subject were usually low. However, the effect of carbon dioxide was not consistent.

Ballard (195) found that at low intensity of radiant energy both sexes of the stable fly were attracted to a source in the region of 365 mμ, 465 and 640 mμ. At high intensity of radiant energy the males showed four response peaks: 390 mμ, 440, 515, and 640 mμ. At this intensity the females responded maximally at 365 and 640 mμ. Infrared seemed to indicate repellency.

Dethier (1957) has reviewed the sensory physiology of blood-sucking arthropods. Krijgsman (1930) recognized four sequences in the responses of the stable fly to stimuli from a mammalian host. (1) a positive taxis to the host, (2) extension of the proboscis, (3) probing or piercing, and (4) ingestion. He also showed that the skin odor of horse, dog, buffalo, and man caused the stable fly to extend it proboscis and to pierce. Moisture, heat, and the odor of fresh horse blood had the same effect.

Schaerffenberg and Kupka (1951) discovered a highly volatile unspecified blood constituent which is attractive to the stable flies. It apparently acts as an attractant and also elicits the act of piercing. When the material was placed beneath animal membranes or single layers of filter paper, the stable fly (and Culex also) were observed

to pierce through the covering and suck the attractant. According to the authors this volatile blood constituent diffuses through the skin and is a most important factor in attracting biting flies to the host.

Hopkins (1964) studied the probing response of the stable fly to vapors in small still air chambers, and demonstrated a strong probing response to the vapors of ammonia. A concentration of 1.1 mg of ammonia per liter of air caused probing in 46% of the flies tested, whereas 75% probed when the concentration of ammonia in the air was doubled. The vapors above 0.1% solution of n-propyl amine and above concentrated n-caprylic, n-caproic, and valeric acid had an effect similar to that of ammonia as probing stimulants. Removal of the antennae or maxillary palpi had no significant effect on the probing response to ammonia but covering the tarsi with lacquer reduced the magnitude of the response.

According to Hopkins (1964) probing precedes biting in the feeding pattern and increases the chances of encountering further stimuli for biting and ingestion. Nevertheless, the stable fly will suck withdrawn blood without first probing or biting so that the latter are not obligatory responses in the feeding sequence. The stimulating effect of the blood on the tarsal and labellar chemoreceptors is sufficient to induce proboscis extension followed by ingestion.

Contact chemoreceptors on the tarsi and the labella of the stable fly have been demonstrated by Adams et al. (1965) and Adams and Forgash (1966). Such chemoreceptors are two-toned, thin walled setae. Fewer contact chemoreceptors occur on the legs of Stomoxys than in all other flies that have been observed, except Glossina palpalis. The number of these sensory setae is greatest in the prothoracic legs and least in the metathoracic legs. The males have more tarsal contact chemoreceptors than the females.

Longevity.-- In nature, the longevity of the stable fly has been estimated at about 3 weeks (Bishopp, 1939). In the laboratory Mitzmain (1913) was able to keep one female alive for 72 days and one male for 94 days. He maintained the flies in the dark, at a temperature of 22 C, fed them daily on monkeys or guinea pigs, and transferred them to clean vials after each feeding. Herms (1961) kept 4,000 flies in glass quart jars, 50 flies to a jar. The average length of life under favorable laboratory conditions and daily feedings on monkeys or rabbits was 20 days.

Population Control by the Sterility Method

The sterile male concept

The sterile male concept is the term applied to the use of sexual sterility to control the population of an animal species. The idea was first conceived by E. F. Knipling as early as 1937, when he deduced that it might be possible to rear and release large numbers of screw-worm flies sterilized by chemicals or radiation, sufficient to overwhelm the natural population (Knipling, 1960). The idea was further developed by workers of the U.S. Department of Agriculture and eventually culminated in the eradication of the screw-worm fly from the island of Curacao W.I. in 1955; from Florida and Southeastern states of the U.S.A. in 1959 and all of the U.S.A. by 1966. Special accounts of these campaigns have been written by Baumhover et al. (1955), Baumhover (1958), Knipling (1960), and Baumhover (1966).

Other successful eradication campaigns were those of the melon fly, Dacus cucurbitae (Coquillet) and the oriental fruit fly, Dacus dorsalis (Hendel) from the island of Rota in the Marianas Islands (Christenson, 1966).

The sterility induction method as discussed by Knipling (1964) states that sexual sterility in insects can be produced in several ways, namely: irradiation with gamma rays; treatment with chemicals, and, conceivably, by hybridization among incompatible varieties or strains of insects. The objective is to produce sterility without detrimental effect on mating competitiveness.

Knipling's computations show that by overflooding succeeding generations of a given natural population with a fixed number of sterile insects, originally at a ratio of 9 to 1, complete eradication should be achieved after four generations. In practice, eradication has, in effect, been attained in short periods of time. For the screw worm it was 14 weeks in Curacao (170 sq. mi.) and 18 months in the Southeastern United States (80,000 sq. mi.) (ARS special report, 1962). The melon fly disappeared from the island of Rota (30 sq. mi.) four months after the releases began (Christenson, 1966).

In the examples given above, radiation-sterilized insects have been released. However, a chemosterilant has already been used to sterilize insects for subsequent release. Reared Mexican fruit flies, Anastrepha ludens (Loew) sterilized with the chemosterilant tepa have been released in northern Mexico, and have performed successfully in limited campaigns aimed only at reducing the natural population (Shaw and Riviello, 1965).

Another promising approach is based on the sterilization of members of the natural population in situ to an extent sufficient to bring about its own destruction. This objective could well be attained by the proper application of chemosterilants. To date such an approach has been tested with partial success in trials aimed at the control of the house fly, Musca domestica (L.) (LaBrecque et al., 1962; LaBrecque et al. 1963; and Gouck et al. 1963) and the Mexican fruit fly, Anastrepha ludens (Loew) (Shaw and Riviello, 1965) through the use of chemosterilant treated baits.

Types of induced sexual sterility in insects

According to LaChance et al. (in press), induced sterility in insects may be due to four principal causes: 1) dominant lethal mutations; 2) aspermia; 3) sperm inactivation and, 4) infecundity.

Dominant lethal mutations.-- Dominant lethal mutations include point mutations, chromosome lesions and chromosome breaks. There may be loss of relatively large blocks of genes through chromosome break and elimination or abnormal distribution of chromosomes or chromsome fragments (Sonnenblick and Henshaw, 1941). The effect of dominant lethals is the death of the zygote at some stage of development so that the insect does not reach maturity.

Dominant lethals can be induced both by irradiation and by treatment with chemicals. Muller (1927), in a classic paper, reported conclusively that treatment of the sperm of Drosophila melanogaster with heavy doses of x-rays induced the occurrence of true gene mutations. Lethals, both dominant and recessive, greatly outnumbered the non-lethals. The overall mutation ratio increased about 15,000 percent over that in untreated germ cells. Sonnenblick and Henshaw (1941) stated that following irradiation of germ cells of Drosophila melanogaster, meiotic and mitotic divisions can become drastically disordered, displaying aberrant chromosomes and achromatic figures. These authors also observed disordered cell proliferation and lack of differentiation in the developing embryo.

Induction of dominant lethal mutations by chemicals has also been demonstrated. Darlington and Koller (1947) state that breakage of chromosomes can be induced by treatment with chemicals such as potassium thiocyanate, ethyleneurethane, sulfur and nitrogen mustards, sulphonamides, allyl isothiocyanate and phenol.

Fahmy and Fahmy (1964), in discussing the chemistry and genetics of the alkylating chemosterilants, state that alkylation of DNA bases may result in mutations and pairing errors. They reported maximal mutagenicity on mature sperm with sulfonic esters; on early spermatids with

the epoxides, ethyleneimines and carboxylic acid mustards; on spermatocytes and early spermatids with the amine mustards and on the spermatogonia with the aminoacid mustards.

According to LaChance et al. (in press), chromosome abnormalities occur in nearly all embryos arising from zygotes where males have been chemosterilized. They list 34 compounds which are only "a few" of those known to produce chromosome breaks in plants and animals. Borkovec (1966) cites 11 references in which chromosomal aberrations have been reported in cells of insects treated with alkylating agents.

Dominant lethal mutations manifest themselves mainly in the death of the embryo (non-hatchability of the eggs) but death may occur in any other stage of development. For example, Borkovec (1966) states that a number of chemosterilants, notably some s-triazine derivatives, fed to adult house flies have moderate or no effect on the egg hatch but the larvae do not reach the pupal stage.

Sterility due to dominant lethal mutations is most commonly produced when male insects are treated with chemosterilants but it can also occur selectively in the females. For example, LaChance et al. (in press) state that some anti-metabolites, such as the purine and pyrimidine analogs, which are directly related to nucleic acid metabolism, are effective only upon chromosomes that are duplicating. Therefore they sterilize the females only, causing mostly point mutations.

Dominant lethal mutations are the type of sterility most widely and successfully used to date in the application of the sterile-male technique of population control (LaChance et al. in press).

Aspermia.-- Aspermia is defined by LaChance et al. as the failure of males to produce sperm at all or failure to continue producing sperm

after the original supply becomes exhausted. Aspermia is the result of damage to the spermatogonial cells. It can be caused by irradiation or by treatment with chemicals.

Cantwell and Henneberry (1963) exposed 3 to 4 day old adults of Drosophila melanogaster Meigen to 8 and 16 Kr of gamma radiation, or fed them 0.25% and 1.0% apholate in sugar-yeast bait for 24 hours. The higher dosages of either treatment caused cessation of sperm production in the anterior end of the testes after the 8th day and progressive necrosis of the germinal epithelium until the 19th day when few sperm were observed.

Borkovec (1966) cites 7 references in which various degrees of testicular atrophy or inhibition of testicular growth has been reported in insects following treatment with alkylating agents. These include effects on the Mexican fruit flies, Drosophila, mosquitoes, eye gnats, and boll weevils. Such effects might result in aspermia. For example, Schwartz (1964) reported that the testes of the eye gnat, Hippelates pusio Loew treated with tepa, metepa or apholate were smaller than those of untreated gnats and showed degeneration in the germarial region.

According to LaChance et al. (in press), aspermia may be a useful form of sterility in species in which the females are monogamous and the transfer of sperm is not a requisite for monogamy. For example, Rieman et al. (1967) found that loss of receptivity by house fly females was primarily due to the male seminal fluid, not to mechanical stimulation or sperm. Of 129 females mated to aspermic males only 14% remated, compared to 7% remating when originally mated to normal males. They concluded that sperm inactivation or even aspermia would not be detrimental in house fly control attempts.

Sperm inactivation.-- Sperm inactivation may be manifested in any one of three ways: (1) loss of motility of the sperm, (2) inability to penetrate the egg, and (3) failure to function in the early stage of embryogenesis. In the first two instances inactivated sperm would not be competitive with normal sperm, while in the third case the sperm would probably be competitive.

Sperm inactivation does not usually result from the dose of irradiation or chemosterilants applied for sterilizing insects, although it may result from higher dosages. Whiting (1938) found that in Habrobracon all sperm had at least one lethal mutation when it was irradiated with 10,000 to 20,000 r. At dosages of 41,000 to 142,000 r, however, some sperm were inactivated but many were still active and presumably able to carry dominant lethals into the egg.

Mendoza (1964) reported that injection of 20 micrograms of apholate did not adversely affect the sperm of the southern corn rootworm (Diabrotica undecimpunctata Howardi), while 40 micrograms or more caused weakening and inactivation of the sperm. On the other hand, LaChance et al. (in press) showed that tepa produced significant inactivation of the sperm in Bracon even at substerilizing doses, while tretamine did not produce sperm inactivation at any level. This suggested that sperm inactivation occurs with certain chemosterilants and is not necessarily due to overdose.

Sperm inactivation is considered undesirable in the sterility method of population control. However, according to LaChance et al. (in press) sterility based on sperm inactivation would be effective when females mate only once and, additionally, sperm transmission is not required for monogamy in the female.

Infecundity.-- Infecundity means failure to produce eggs. Such failure may result from many different causes, since fecundity itself is the end product of many interrelated physiological processes in the female.

According to LaChance et al. (in press), infecundity may result from 1) death of gonial cells; 2) non-function of the nurse cells and, 3) interruption of any of the steps of vitellogenesis by environmental, hormonal, biochemical or genetic factors.

In most cases where induced infecundity has been observed in insects, it has been found that the ovaries are poorly developed. That is, that ovarian growth has been stopped or greatly inhibited as a consequence of the treatment applied to the female. This type of action has been observed in insects that have been irradiated with gamma rays or treated with chemosterilants in the late pupal stage or as young adults.

Cantwell and Henneberry (1963) treated 3 to 4 day old adults of Drosophila melanogaster Meigen with 16 Kr gamma radiation or 1% apholate given for 24 hours in a sugar yeast bait. The ovaries of treated females were reduced in size and by the 10th day after treatment very little ovarian tissue remained. Histological examination suggested that complete breakdown of the nurse cells, oocyte and follicle cells had occurred.

According to Borkovec (1966), retardation or complete cessation of ovarian development by chemicals has been observed in house flies (9 references), Mosquitoes (3 references), various fruit flies (8 references), face flies, eye gnats and Habrobracon wasps (1 reference each). Morgan and LaBrecque (1962) reported that apholate 1% when given in the food to adult house flies for a period up to 240 hours starting the day of emergence inhibited but did not eliminate ovarian development. There was damage to the nurse cells of the first and second egg chambers and

to the germarium. The ovaries of treated females increased in size up to 2.435 mm^3 compared to 4.208 mm^3 in the untreated flies.

Crystal and LaChance (1963) found that 0-4 hour old female screwworm adults treated with a benzoquinone compound and with methyl tretamine later failed to lay any eggs. The ovaries of the benzoquninone treated flies were all very small, 50-75% of them were less than 1 mm^3. Those of flies treated when they were 1 day old varied widely in size. Ovaries of untreated flies were generally greater than 7 mm^3.

Morgan (in press) found that female house flies maintained on food containing 1 or 2% hempa developed eggs from the first egg chamber, but no eggs were fully developed from the second and third egg chambers of the ovarioles. Ovarian development was inhibited.

Infecundity in insects is not invariably associated with underdevelopment of the ovaries. Simkover (1964) found that 1% 2-imidazolidinone administered in a milk diet to female house flies during the first 5 days of adult life completely prevented oviposition. However, he did not observe any marked difference in ovarian development between treated and untreated individuals. Similar treatment of male house flies did not show any sterilizing effect.

In most cases induced infecundity by different kinds of chemicals has been reported without reference to any physiological effect in the female. LaChance et al. (in press) list a wide variety of compounds which reduce oviposition in insects. Included are antimetabolites, alkylating agents, insecticides, acaricides, herbicides, antivitamins, antihelminthics and even blood anticoagulants.

Infecundity may be an important form of sterility in the control of populations by the sterility method. Reference is generally made

to the "sterile-male release technique" but in every successful use of the method both sterile males and females have been released. Husseiny and Madsen (1964) experimented with the navel orangeworm Paramyelois transitella (Walker) and showed that, at least in particular cases, the release of sterile females alone might even be better than releasing only males. Ailam and Galun (1967) also showed mathematically that the introduction of sterile individuals of the two sexes is never inferior, and sometimes is even superior to the introduction of one sex alone. LaChance et al. (in press) state that in species with shortlived males the release of sterile females might have a significant effect on the population trend.

Chemosterilants

Types of chemosterilants

The term chemosterilants was first introduced by LaBrecque et al. (1960) to designate chemical compounds which induced various degrees of sexual sterility in insects. A large number of such chemosterilants are now known as a result of intensive screening of thousands of chemicals, since 1958 in laboratories of the U.S. Department of Agriculture. (LaBrecque, et al. 1960; Lindquist, 1961; Crystal, 1963; Fye et al. 1965). Extensive reviews on insect chemosterilants have been prepared by Smith et al. (1964); Borkovec (1966) and Smith and LaBrecque (in press).

Insect chemosterilants comprise compounds of very diverse chemical composition, but two main groups have gained prominence because of their consistent effectiveness. These are the biological alkylating agents and the antimetabolites. All others that do not fall in these two broad categories are classed as miscellaneous chemosterilants. Borkovec (1966)

lists 405 chemosterilants shown to be effective upon 50 species of insects, 5 species of mites and 2 species of ticks. The list includes 196 alkylating agents, 49 antimetabolites and 160 miscellaneous compounds.

Alkylating agents

Crystal and LaChance (1963) define a biological alkylating agent as "a compound that can effect the addition of an alkyl group or a compound radical, with or without the replacement of a hydrogen atom, in biologically significant functional groups under physiological conditions." The biological alkylating agents have been extensively reviewed by Ross (1962) and Whitelock (1958) and generally discussed, relative to their antifertility effects, by Jackson (1966) and Borkovec (1966) among others.

According to Borkovec (1966) three main classes of biological alkylating agents have attained prominence as insect chemosterilants, namely: the ethyleneimines (aziridines); the 2-chloroethylamines ("nitrogen mustards"); and the sulfonic acid esters (alkyl alkanesulfonates). Ethyleneimines are the most important and numerous group. They comprise various aliphatic, aromatic and heterocyclic analogs of ethyleneimine, and possess outstanding chemosterilant activity (Crystal and LaChance, 1963).

Ethyleneimines characteristically have one, two or more aziridine groups attached to a carrier molecule, and accordingly are classed as monofunctional, bifunctional or polyfunctional. The aziridine group and its variants are recognized as the active parts of the molecule but the relationship between the number of such functional groups and sterilant effectiveness is not yet established.

Crystal (1966) has analyzed the results of screening tests with 200 aziridinyl compounds of known structure as sterilants of the screw-worm fly. The compounds were grouped according to mode of administration

(topical or multiple oral); substitution on aziridinyl carbon (substituted or unsubstituted); and number of functional radicals (mono-, bi- and polyfunctional). Among the unsubstituted compounds there was no difference of effectiveness attributable to the number of functional groups when tested either as a single topical treatment or as multiple oral treatments. However the multiple oral treatment, as a screening method, was substantially superior to the single topical application.

Among the substituted compounds there was no difference ascribed to the number of functional groups by the multiple oral treatment. However, when the chemicals were tested topically a larger proportion of polyfunctional compounds induced sterility than of mono- or bifunctional compounds.

Crystal states also that his study confirms well known evidence that the presence of substituents on the aziridine carbon atoms reduces the chemosterilizing ability of a compound. Toxicity of compounds to the screw-worm flies was largely unrelated to degree of functionality, to substitution and only partly related to mode of administration. Generally speaking it can be said that the biological properties of alkylating agents depend on the number of alkylating groups and the type of carrier molecule (Jackson, 1966). However their exact mode of action in causing sterility is not known and no single theory can as yet be formulated (Borkovec, 1966).

Some of the best known insect chemosterilants that belong to the aziridinyl class are: tepa, metepa, tretamine and apholate. Tepa has been reported as effective upon 23 species, Metepa upon 21 species, Tretamine upon 7 species and apholate upon 32 species of insects and mites (Borkovec, 1966). The first 3 mentioned are trifunctional, and the last (apholate)is hexafunctional.

Antimetabolites

Antimetabolites can be defined as compounds which are antagonistic analogs of metabolites. They are chemically and structurally similar to important metabolites and are thought to be able to replace or displace such metabolites, thereby disrupting the metabolic process (Borkovec, 1962).

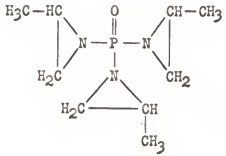
According to Crystal (1963) two main groups of antimetabolites can be distinguished in regard to insect chemosterilization: 1) Compounds presumably affecting de novo synthesis of purines and pyrimidines. In this group are folic acid antagonists, such as amethopterin and aminopterin, and glutamine antagonists, such as azaserine. 2) Compounds presumably affecting incorporation of purines and pyrimidines into nucleic acid. These include purine and pyrimidine antagonists, such as 5-fluorouracil and 5-fluoroorotic acid.

Antimetabolites are notable as female insect chemosterilants and their main effect is infecundity. As early as 1952, Goldsmith and Frank found that aminopterin reduced or prevented oviposition in Drosophila, and later Mitlin et al. (1957) recorded this effect on the house fly. Amethopterin completely prevented oviposition in the house fly (LaBrecque et al. 1960) and in the screw-worm fly (Crystal, 1963). To date aminopterin has been effective on 7 species of insects; amethopterin on 9 species; 5-fluorouracil on 8 species, and 5-fluoroorotic acid on 3 species (Borkovec, 1966, Painter and Kilgore, 1964).

Antimetabolites may also have sterilizing effects on male insects. For example, Crystal (1963) found by topical application that 6-diazo-5-oxonorleucine, a glutamic acid antagonist, sterilized only the males in the screw-worm fly.

Metepa

Metepa is a trifunctional aziridinyl derivative having the following structural formula:



Metepa's chemical name is Tris (2-methyl-1-aziridinyl) phosphine oxide but is also known by several other designations, namely: Methaphoxide, MAPO and U.S.D.A. Ent. 50003.

According to Interchemical Corporation (1962) the physical properties of metepa are as follows: Straw-colored liquid having an odor of high boiling amine. Boiling point at 1 mm Hg 118-125°. Specific gravity $25°/25°$ 1.079. Refractive index n_d^{25} 1.4798. Completely soluble in water and all common organic solvents. Available in a formulation containing 92% metepa based on reactive imine assay and no more than 0.5% volatile material. This formulation has excellent storage stability at room temperature.

Metepa degrades very rapidly under acidic conditions. Borkovec et al. (1964) found that aqueous solutions of metepa maintained at pH 3.8-4.2 for periods of time ranging from 0 to 180 minutes very rapidly lost their sterilizing effect upon male house flies. The percent sterility dropped from 94% for the 0-minute old solution to 0% for the 180-minute old solution. The sterilizing effect was found to be proportional to the content of intact metepa, rather than to the total content of aziridine.

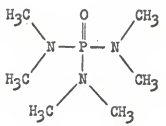
However, metepa reacts very slowly with water under alkaline or neutral conditions (Interchemical Corp., 1962). For example, starting with a 0.3 aqueous solution of metepa, negligible decomposition had occurred after 20 days at 3 C and only 10% had decomposed at 25 C. However, 45% of metepa had disappeared at 50 C after only 12 days (Beroza and Borkovec, 1964).

As a chemosterilant metepa is classed among the more outstanding alkylating agents. It has been reported effective upon 21 species of insects (Borkovec, 1966). It is effective upon males and females, usually requiring lower dosages in the males to produce complete sterility (Harris, 1962).

Metepa is toxic to warm-blooded animals. In rats the oral LD_{50} in a single dose is 136 mg/Kg and the dermal LD_{50} is 183 mg/Kg. Repeated daily oral dosages of 5 mg/Kg given to male rats produced marked infertility in 22 days, complete sterility within 70 days and testicular atrophy within 77 days. One out of 12 rats died in 89 days. (Gaines and Kimbrough, 1964).

Hempa

Hempa is a phosphoric triamide derivative having the following structural formula:



Hempa's chemical name is tris (dimethylamino) phosphine oxide but it is also known by several other designations, namely: Hexamethylphosphoric triamide, hexamethylphosphoramide, HMPA, and USDA Ent. 50882.

The physical properties of hempa, as given by Turner (1967 in press) are as follows: Colorless liquid having mild amine odor. Melting point 6-8 C; boiling point 230-232 C at 739.4 mm Hg or 70-72 C at 1-1.5 mm Hg. Refractive index n_d^{20} = 1.4586-1.4590. Infinitely soluble in water and all common plasticizers and in both polar and non-polar solvents. Stable under normal storage conditions. Chemically hempa is remarkably stable, resistant to alkaline hydrolysis and to dilute acids (Borkovec, 1966).

As a chemosterilant hempa is classed among the miscellaneous compounds. It is structurally similar to tepa but lacks alkylating properties (Chang et al. 1964). It is effective mainly on males, its effect on females being rather erratic (Chang et al. 1964). The sterilizing action of hempa has been ascribed to mutagenesis (Palmquist and LaChance, 1966).

Hempa is a chemosterilant with low toxicity for mammals. Adkins et al. (1955) reported the highest sublethal dose in rabbits as 1,300 mg/kg. and the lowest lethal dose as 1,500 mg/kg. In their experiments hempa was tested as a systemic insecticide. When fed to rabbits at 1,300 mg/kg. it caused 63% mortality of bed bugs and 100% mortality of the lone star tick. Kimbrough and Gaines (1966) found that the acute oral LD_{50} in rats is greater than 2,500 mg/kg. and the acute dermal LD_{50} greater than 3,500 mg/kg. A daily dose of 40 mg/kg. administered to males caused testicular atrophy and reduction of fertility in 45 days. Females tolerated a daily dose of 200 mg/kg. for 45 days.

The use of chemosterilants in the sterility method

Chemosterilants could be used to the greatest advantage in treating a proportion of the natural population in situ. Smith et al. (1964)

have discussed the advantages accruing thereby. Treatment of the natural population would make it unnecessary to rear large numbers of insects for subsequent release thus significantly reducing the cost of population control. Moreover the method would be applicable to species which are destructive or otherwise harmful if released in large numbers, e.g., vectors of disease.

Knipling (1962) has shown that sterilizing treatments would be more effective than insecticidal control. A constant pressure arising from sexual sterility in 90% of the population would eradicate the species on the 5th generation, while it would require 20 generations to attain the same goal by constant insecticidal pressure causing 90% kill.

Lindquist (1961) has suggested the possibility of applying residual treatments to such places as vegetation near swamps, where horse flies, deer flies and biting gnats breed or congregate; or else the use of a bait or lure for feeding stations. He suggested that the chemosterilant could also be combined with an insecticide and insects not killed outright would become sterile. These would disperse and help control insects not reached by the treatment. Also the inheritance of resistance to insecticides would likely be impaired.

The most important limitation on the use of chemosterilants resides in their mutagenic properties and their generally high toxicity. Fahmy and Fahmy (1964) have discussed the genetic hazards to man. They state that the "doubling dose," i.e. the dose required to double spontaneous mutation rates in a human population can be estimated at 35 r of ionizing radiation per generation. This would mean one additional mutation per 5 individuals per reproductive generation (30 years). Genetic extinctions through death or sterilization would occur and their frequency per

generation after the doubling dose is expected to be one incident per 200 individuals. According to the authors this would mean some 250,000 genetic extinctions per generation in the population of the United Kingdom alone.

The "doubling dose" of chemosterilants is given by Fahmy and Fahmy as a minimum of 0.1 mg/kg for strong mutagens, such as TEM (tretamine) to a maximum of 20 mg/kg for weaker mutagens like myleran. The authors concluded that the use of such strong mutagens for insect control, especially when this necessitates their spraying in human dwellings, would have effects as devastating on our genetic heritage as would a large scale atomic war.

The toxicology of chemosterilants has been reviewed by Hayes (1964) and Hayes (in press), who states that most promising chemosterilants are known to be acutely toxic to warm blooded animals at relatively small doses. However, the possible effects of repeated small exposures are unknown, although the action of the few compounds that have been studied proved to be cumulative.

Hayes (1964) notes that some of these compounds have a moderate acute toxicity compared with many insecticides, while some are highly toxic. Tretamine, for example, has an oral LD_{50} of 1 mg/kg in the rat; similar to that of mustard gas or to the insecticide tetraethylpyrophosphate (TEPP). Hayes further notes that animal species differ in sensitivity, as exemplified by sheep which have been shown to be much more sensitive than rats to apholate.

On the other hand alkylating agents, notably the aziridines, are highly reactive so that danger of prolonged contamination of the environment is reduced (Borkovec, 1966). Beroza and Borkovec (1964) showed

that aziridine chemosterilants, i.e. tepa, metepa, and tretamine were highly sensitive to even mildly acidic conditions. Chang and Borkovec (1966) employing bioassay and colorimetric methods, determined that 90% of tepa had disappeared in three days from the body surface of topically treated Mexican fruit flies; so that 8-9 day old flies (the average age at which they were being released in infested areas in Mexico) probably did not bear any detectable residues of tepa.

The use of baits and lures for selective treatment of insect species also offers an avenue for carefully controlled application of chemosterilants. The target insect can be attracted to the station, where it would remain temporarily, becoming permanently sterilized in the process. Borkovec (1966) cites 7 field experiments conducted for the control of natural populations of house flies by means of baits treated with tepa, metepa, or apholate. He also described a plastic bait station used by Shaw and Riviello to attract and chemosterilize the Mexican fruit fly in a mango orchard.

Chemosterilization of the stable fly

To date 6 reports have appeared in the literature regarding effects of chemosterilants on the stable fly. Borkovec (1962) reported that normal females of the stable fly, mated to apholate-treated males, laid a normal number of eggs which had low viability; on the other hand mating of treated females with untreated males resulted in reduced fecundity. The eggs laid, however, had normal viability.

Harris (1962) tested apholate, tepa and metepa by topical treatment on the stable fly. One microgram of apholate or metepa per fly caused almost complete sterility when treated males were mated with treated females. Apholate was effective even when applied to flies 1 to 7 days

old. When only one sex was treated with apholate complete sterility was not attained even at 7.4 micrograms per fly. Apholate also induced sterility when both sexes were exposed to a film of 10 mg per 1/2-pint jar for 48 hours; or to a film of 100 mg per 1/2-pint jar for one hour. When treated jars were stored indoors the film remained effective for 24 weeks.

Harris (1962) noted that the female stable fly was much more susceptible to apholate than the female screw-worm fly. The latter required 150 micrograms of apholate topically applied for 90-100% control of reproduction (Chamberlain, 1962), compared to 3.7 micrograms in the female stable fly. Chamberlain and Barrett (1964) compared the susceptibility of the stable fly and the screw-worm fly to metepa. Topically the male screw-worm fly required 5.5 times more metepa per gram of body weight than did the male stable fly. The corresponding values for feeding treatments for the screw-worm fly and the stable fly were 3.9 and 6.2 times, respectively for the males and the females.

Chamberlain and Hamilton (1964) sought to explain such difference in susceptibility. Using P32-labeled metepa they found that 6 hours after treatment the screw-worm fly absorbed only half as much radiolabeled metepa in proportion to its size as did the stable fly. Excretion of radioactive material by the screw-worm fly was twice that of the stable fly, but the metabolism was half as fast as in the stable fly.

Simkover (1964) evaluated 2-imidazolidinone as a growth inhibitor and chemosterilant on several insects. The compound incorporated in the larval medium at 330 ppm and 660 ppm inhibited larval development in the stable fly. The treatment resulted in the formation of characteristically misshaped pupae, which failed to produce adults.

Parish and Arthur (1965a) studied the metabolism of thiotepa in rats and in 4 species of insects, including the stable fly. Thiotepa was applied topically at a dose of 100 mg/kg. In the stable fly maximum absorption (81.2%) had occurred 4 hours after treatment. The amount of thiotepa decreased with time after treatment. Such decrease was apparently due to elimination of the thiotepa in the feces.

MATERIALS AND METHODS

The Fly Colony

Source

The stable flies used in these investigations were from a colony originally obtained from the Entomology Research Division, Agricultural Research Service, U.S.D.A. Kerrville, Texas, and maintained in the U.S. Department of Agriculture Entomology Research Laboratory for Insects Affecting Man and Animals located at Gainesville, Florida.

The colony was maintained by the author following the rearing method currently in use at the Laboratory with several minor modifications developed in the course of this work. A detailed account of the method of rearing follows:

Rearing the larvae

The larvae were reared in a medium composed of one liter of CSMA Fly Larval Medium*, one liter of clean white pine wood shavings, and 1.25 liter of distilled water. The ingredients were thoroughly mixed and put in an 8 liter glass jar (21 cm diameter x 28 cm deep). For the first few batches the medium was placed in a polyethylene bag, which was put inside the jars as a convenience in handling the spent medium, but it was found this was not necessary (Fig. 1).

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* Obtained from Ralston Purina Company. According to Jones (1966) CSMA fly larvae medium consists of 26.67% alfalfa meal, 33.33% soft wheat bran, and 40% brewer's dried grain.

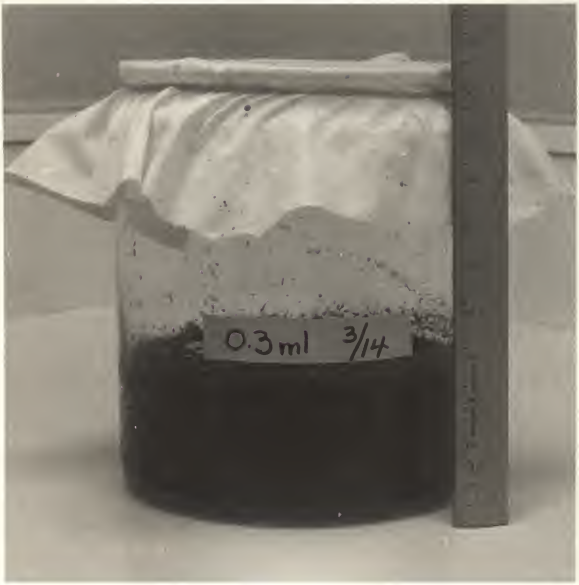

Fig. 1.--Rearing jar containing culture of stable fly larvae in a medium composed of 1 liter of CSMA fly larval medium, 1 liter of wood shavings, and 1.25 liters of distilled water.

Clean wood shavings of the right kind of wood were necessary for proper rearing. Best results were obtained by using white pine shavings. White pine boards were converted to shavings in the Laboratory shop. Mixed shavings, mostly oak, obtained from a local mill were tried but were not always satisfactory. Sometimes the larvae seemed to be repelled by the medium prepared with such shavings. They would crawl out of the medium and up the walls of the jar, where they became desiccated and died. The medium was seeded with 0.3 ml of eggs placed on the surface of the medium and washed in with a small amount of water. The jars were then covered with cotton cloth held in place with a rubber band. Throughout these operations care was always taken to avoid contamination with other kinds of fly eggs.

The rearing room at the Gainesville laboratory is air conditioned and maintained under constant light at 26.5 C and 55% relative humidity. If the medium is properly prepared with the right proportion of water, no overheating or drying of the medium occurred during the rearing period.

Pupation occurred 9 to 10 days after seeding and the pupae were found just under the surface of the medium, often congregated around the periphery. On the 12th day the top layer of the medium containing the pupae was removed and placed in a pan of water. The pupae, which floated to the surface, were then taken out and gently washed free of debris. The cleaned pupae were air dried over a wire screen frame. The air dried pupae were finally put in waxed paper cups covered with paper toweling and stored in the refrigerator at 5.6 C. Pupae could be kept up to 7 days under this condition without impairing emergence. About 2,000 pupae were regularly obtained from each jar.

Production of eggs

In the production of eggs about 2,000 pupae were put inside a cylindrical screen wire cage 30 cm diameter x 45 cm in length with a circular opening at one end, to which a cloth sleeve was attached. The adults emerged on the 6th and 7th day after pupation (i.e. 4 days after floating) and immediately were provided with citrated bovine blood (100 ml of 12% aqueous solution of sodium citrate added to 4 liters of blood) by means of soaked cotton balls placed daily on top of the cages. Such citrated blood was kept under refrigeration but it was not necessary to warm it before feeding the flies (Fig. 2).

Usually on the 6th day after emergence egg masses were observed on the underside and edges of the blood soaked cotton balls. Eggs were collected by providing the flies with a proper oviposition medium, which consisted of the moist rearing medium remaining after removing the pupae. This medium gives off a strong ammonia odor which apparently stimulates oviposition. At times freshly mixed medium to which a small amount of ammonium hydroxide had been added was also used effectively. A waxed paper cup was partially filled with the oviposition medium, which then was covered with a well moistened, wrinkled black cloth. The cup containing the oviposition medium was left inside the cage for a period of one or two hours.

The eggs were gently washed off the cloth into a clean waxed paper cup and measured into graduated centrifuge tubes by means of a medicine dropper. In this way 2 ml of eggs could be collected daily for 4 days. After that the fly population rapidly declined. Six days after the start of oviposition the population was discarded.

Fig. 2.—Cylindrical wire screen cage (30 cm diam x 45 cm long) containing about 2,000 adult stable flies. Cotton balls soaked in citrated bovine blood were placed daily on top of the cage to feed the flies.

Blood for feeding

Bovine blood used for feeding the stable fly was obtained at a local abattoir in clean glass jugs which contained 100 ml of a 12% aqueous solution of sodium citrate. The blood and the sodium citrate solution were thoroughly mixed by inverting the bottle a number of times. This blood, which is referred to as citrated blood, was stored in the closed containers at 2 C for periods up to 5 weeks without apparent loss of nutritive value.

Chemosterilant Studies

Separation of sexes

As it became necessary sexes were separated while the adults were immobilized by chilling. Chilling was preferred to CO_2 as being less harmful to the flies. Harris et al. (1965) found that chilling flies at 4 C even for periods up to 6 hours would result in only 6-9% mortality, as compared to 25% mortality for CO_2 after only one hour exposure. In these experiments sexes were separated inside a cold room maintained at about 2 C. Exposure to this temperature immobilized the flies in 3 to 5 minutes. Only small lots of about 100 flies were sexed at one time to minimize their exposure to the cold (about 15 minutes).

The sexes were easily distinguished visually by the size of the black spot delimited by the genitalia and an adjacent ventral spot at the tip of the abdomen. In the male the combination of the two spots appear characteristically shaped and are relatively large and conspicuous, while it is hardly visible in the female (Figs. 3 and 4).

Laboratory environment

Except where otherwise noted all the experiments were conducted

Fig. 3.--Ventral view of male stable fly showing large, conspicuous black genitalia and adjacent ventral spot at the tip of the abdomen. 14 X.

Fig. 4.--Ventral view of female stable fly showing small, inconspicuous black spot, the genitalia, at the tip of the abdomen. 15X

in the laboratory under a controlled environment maintained at 26.6 C and 55-60% relative humidity under a 12 hour photoperiod.

Chemosterilants

Commercial grades of metepa (92%) and hempa (100%) were used for the preparation of the aqueous solutions needed in these experiments. The solutions of the chemosterilants at the required concentrations were prepared by an experienced technician of the chemistry section of the laboratory.

Decontamination of equipment

Decontamination of durable cages used for chemosterilant studies was accomplished by washing them first in warm, soapy water, to which about 10% of vinegar had been added and then rinsing them with cool tap water. Glass tubes used for treated blood were decontaminated by first washing them in soapy water, then leaving them immersed in full strength vinegar for 12 hours and finally rinsing them in cold tap water.

Non-durable materials, such as cardboard cages, paper cups and the like were discarded after being used only once.

Statistical methods

Dose-sterility and dose-mortality curves were constructed and analyzed by the methods of Lichtfield and Wilcoxon (1949) and the longevity data were analyzed by the methods given by Snedecor (1956) regarding comparison of sampled populations. Data recorded as percentage of sterility or percentage of mortality were adjusted by means of Abbott's formula (Abbott, 1925).

Sterility Studies

A series of 12 experiments were conducted to evaluate the effect

Fig. 5.--Method used to transfer virgin female (or male) stable flies to cages containing adults of the opposite sex. Fully fed flies were collected from the large cage with a plastic vial and transferred to the smaller cages.

and the range of effectiveness of metepa and hempa as chemosterilants when administered orally to adult male and female stable flies.

Samples of 10 newly emerged virgin males or females were placed in small cardboard cages, fashioned from waxed cardboard ice cream tubs of one liter capacity (Fig. 5), and provided with blood treated with the chemosterilants for 1, 2 or 3 days. The treated blood contained metepa at concentrations ranging from 0.0007% to 0.0500% or hempa at concentrations ranging from 0.015% to 0.500% by weight. These concentrations were obtained by mixing one part of known aqueous stock solutions of the chemosterilants with three parts of citrated bovine blood. The stock solutions were freshly prepared at the start of each experiment and were kept under refrigeration for the duration of the treatment (a maximum of 3 days) to minimize decomposition of the chemosterilants.

The blood was provided to the flies by means of glass tubes (5 mm x 10 cm) fitted with suction bulbs. The tubes were inserted through 6 mm holes at the top of the cages and suspended by their rubber bulbs. One such tube containing about 1.5 ml of blood was sufficient to feed the flies in a cage for one day. The flies were fed once a day, early in the morning, with either treated or untreated blood as the experiments required. There was no need to warm the refrigerated blood, as the flies fed readily on the cold blood.

The treated blood was made available to the flies on the day of emergence. Following the specified exposure to the sterilant the flies were offered untreated citrated blood for 24 hours to insure that all treated food had been excreted. After the 24 hour holding period an equal number of normal virgin adults of the opposite sex

were introduced into each cage (Fig. 5). These adults were of the same age and from the same population as the treated flies and had been fed only citrated blood. Eggs were collected twice. The first collection was made when the flies were 8 days old and the second collection was made 4 days later.

Eggs were collected by placing a small waxed paper cup containing oviposition medium in each cage. The oviposition medium consisted of spent CSMA rearing medium used for rearing larvae of the house fly. A small amount of such medium was wrapped in a piece of well moistened black cloth and wrinkled to produce crevices that served as oviposition sites (Fig. 6). The medium was left inside the cages for 3 to 6 hours. At the end of the exposure period the medium was withdrawn from the cage and the eggs were gently washed off the black cloth into a glass, having a concave bottom. When sterility in the males was being evaluated a random sample of about 100 eggs was removed with a medicine dropper, placed on a small strip of wet black cloth and counted. The eggs were then placed on fresh rearing medium in a waxed ice cream container (1/2 liter capacity) lined with a plastic bag (Fig. 7). The strip of cloth bearing the egg sample was placed face down just under the surface of the medium in order to overcome any rapid drying of the surface and to maintain proper moisture conditions during the 30 to 48 hours required for hatching. The container was then covered with cloth and labeled.

When several containers were used the two main problems were the drying of the medium and overheating. Drying was prevented by placing wet paper towels over the containers during the first 5 days of rearing. Overheating was avoided by leaving a space of at least 2 cm between

Fig. 6.—Method used for the collection of eggs of the stable fly. Eggs were collected by placing a cup containing oviposition medium wrapped in a wet black cloth inside the cages for a period of 3 to 6 hours.

Fig. 7.--Cardboard containers, 1/2 liter capacity, used for rearing larvae from samples of up to 125 stable fly eggs. The containers were lined with a plastic bag and filled with larval rearing medium.

containers. The containers were held inside a screened cabinet to prevent other flies from ovipositing upon the cloth covers. Pupation counts were made ten days after collecting the eggs. Such counts were the basis for evaluating sterility in the males.

In evaluating the sterility of the females all of the eggs were collected and placed on a piece of wet black cloth, which in turn was put inside a petri dish to await hatching. Counts of hatched and unhatched eggs were made 72 hours later. The percentage of sterility in the females was determined by the formula given by Crystal (1965), namely: 100(1-fh). In this formula f (fecundity) is the ratio between the number of eggs laid by the treated females and the number of eggs laid by the control females, and h (hatchability) is the ratio between the proportion of hatched eggs in the treatment and the proportion of hatched eggs in the control.

Longevity Studies

An experiment was conducted to determine the effect, if any, that a dose of metepa or hempa sufficient to sterilize the males, had upon the longevity of treated stable flies.

Samples of newly emerged stable flies, consisting of 40 males plus 40 females, or of 80 virgin females or males alone, all from the same population, were confined in aluminum frame cages 15 x 22 x 25 cm covered with cotton gauze (Fig. 8). The newly emerged flies were provided with bovine citrated blood containing metepa (0.0125%) or hempa (0.125%) for a period of three days, and thereafter were allowed to feed on citrated blood only until the termination of the experiment.

Fig.8..--Aluminum frame cage 15 x 22 x 25 cm used for populations of up to 100 adult stable flies. The flies were provided with citrated bovine blood by means of glass tubes 5 mm diam x 10 cm long, suspended from the top of the cage by their suction bulbs.

Dead flies were removed from the cages and their number was recorded daily. Survivorship curves were constructed and the average lifespan of the flies calculated. The data were compared to those of flies that fed on citrated blood only throughout the experiment.

The flies inside the cages were subjected to a light intensity of about 376 lux (35 foot candles) and under a 14 hour photoperiod. The total population per cage was 80 individuals and each treatment was replicated three times.

Mortality Studies

Four mortality tests were carried out on the stable fly to aid in determining the "Safety factor" of both metepa and hempa as chemosterilants of the stable fly, and thus to have some indication of their practical usefulness. The safety factor is a measure of the dose margin that the insect can receive in excess of the sterilizing dose without apparent deleterious effects. It is obtained by comparing the minimum sterilizing dose with the maximum tolerated dose.

Groups of 100 newly emerged male or female stable flies were placed in aluminum frame cages 15 x 22 x 25 cm covered with cotton gauze, and allowed to feed on citrated bovine blood treated with metepa or hempa at dosages ranging from 0.125% to 0.750% by weight, i.e. dosages estimated to cause mortality.

The flies treated with metepa were provided with the treated blood for a period of 3 days and thereafter they were offered citrated blood only. The mortality count was taken 24 hours after the termination of the treatment period. The flies treated with hempa underwent a one day treatment only, as the mortality counts on the second day were already high enough to establish a dose-mortality relationship.

The safety factor (S.F.) for each chemosterilant was computed on the basis of the respective dose-mortality curves, by adapting the formula employed by Chang and Borkovec (1966a), namely:

$$S.F. \quad \frac{= LD\text{-}.01 - SD\text{-}99.99}{SD\text{-}99.99}$$

where LD-.01 = dose expected to produce .01% mortality in the population tested.

SD-99.99 = dose expected to produce 99.99% sterility in the population tested.

In these experiments the respective values for LC-.01 and SC-99.99 were substituted for the LD and SD values.

where LC-.01 = Percent concentration expected to produce .01% mortality in the population tested.

SC-99.99 = Percent concentration expected to produce 99.99 sterility in the population tested.

Studies on Mating Competitiveness

Two experiments were conducted to ascertain the mating ability of sterilized male stable flies in competition with normal, untreated flies. Only metepa sterilized males were used, as its safety factor (1.56) indicated that there was ample margin between the minimum sterilizing dose and the maximum tolerated dose.

Groups of 100 newly emerged male stable flies, confined in aluminum frame cages 15 x 22 x 25 cm, were sterilized by a three-day treatment with metepa at 0.0125% in citrated bovine blood and allowed to feed one more day on citrated blood only to insure that all the treated food had been excreted. At the same time other groups of 100 males or females of the same population were equally confined and provided with citrated blood only.

On the fifth day different ratios of treated and normal males were put together with normal females in large wooden frame wire screen

cages 60 x 60 x 60 cm (Fig. 9). The total population was fixed at 100 per cage. At this population density the large cages provided sufficient space for adequate interplay between individual flies. The following ratios were used:

Treated males	Normal males	Normal females	Total per cage
33	33	33	99
50	25	25	100
25	50	25	100
50	0	50	100
0	50	50	100

The males, both treated and normal, were counted while they were under anesthesia by chilling and later were put simultaneously in their respective cages. There they were left undisturbed for two hours to allow them to recover from chilling and to familiarize themselves with their new environment. The females were added at the end of this holding period.

All eggs were collected daily from each cage during four consecutive days, starting on the day following the mixing of the sexes. The techniques used for the collection of eggs were the same as those described for the sterility trials, except that the eggs were not put in rearing medium, but in petri dishes only to await hatching. Counts were made after 72 hours and the numbers of hatched and unhatched eggs were recorded and compared with the numbers expected based on the ratios of sterile to normal males.

The citrated blood for feeding the flies in these large cages was provided by means of blood soaked cotton balls placed in waxed paper cups on the floor of the cage. A preliminary trial had indicated

Fig. 9.--Large wooden frame wire screen cage 60 x 60 x 60 cm used for populations of 100 adult stable flies in studies on mating competitiveness. Large volume of cage allows adequate interplay between sexes.

that the use of glass tubes to dispense the blood for feeding was not satisfactory in these large cages because the flies did not seem to find the blood readily.

RESULTS AND DISCUSSION

Sterility Studies

As previously stated the sterility studies consisted of the separate treatment of male or female stable flies, with either metepa or hempa in citrated bovine blood administered orally for one, two or three days.

The results of the treatments for males appear in tables 1 to 8 and the respective dose-response curves appear in figures 10 to 17. The results pertaining to females, in turn, appear in tables 9 to 14.

Metepa induced complete sterility in male stable flies when provided to newly emerged adults at concentrations of 0.0125% for three days, 0.025% for two days, or 0.05% for one day. The concentration for complete sterility in the females was 0.05% for either one, two, or three days' treatment.

Hempa induced complete sterility in the males at concentrations of 0.125% for three days, 0.250% for two days, or 0.50% for one day. The concentration for complete sterility in the females was 0.375 for either one, two, or three days.

When males treated with metepa or hempa were mated to untreated females a proportion of the resulting progeny failed to reach the pupal stage. Such response proved amenable to probit analysis. In each case the concentration required to produce 50% sterility (SC-50) or 90% sterility (SC-90) was determined. These values appear in tables 4 and 8.

Inspection of the dose-response curves reveals that in the case of metepa a one day treatment may not be sufficient to give a reliable response. Based on the potency ratio between the one day and the two day treatments the SC-50 levels do not differ significantly. However, the slope of the line for the one day treatment is appreciably lower, so that the potency ratio at the SC-90 level is considerably greater.

A possible explanation for this result is that at the higher concentrations metepa might have a repellent effect, therefore decreasing the acceptability of the treated blood to newly emerged flies. The flies not engorging during the first day of treatment need to take higher concentration of the chemical for a result similar to the two day treatment.

The dose-response lines for the two and three day treatments with metepa are parallel, the potency ratio being 1.575 at all levels. This signifies that the response of the male stable flies after the second day of treatment shows a reliably uniform dose-effect relationship. It also indicates that there is a significant difference in the effect of a certain concentration of the chemical when it is provided during two days or three days. The potency ratio was statistically significant at the .05 level of probability.

In the case of hempa the dose-response curves for the one, two, or three day treatments are all parallel and are positioned sufficiently far apart so that there is a significant difference in the effect of a given concentration when it is made available for one, two, or three days. The potency ratios are 1.98 between the one and two day treatments, and 1.45 between the two and three day treatments. Both potency ratios were statistically significant at the .05 level of probability.

There was no indication of a repellent effect of hempa at the higher concentrations used. A reliably uniform dose-effect relationship was observed even in the one day treatment.

The sterility induced by both metepa and hempa in the females consisted mainly of infecundity and, to a very minor extent, non-viability of the eggs laid. The response, however, did not follow a regular dose-effect relationship and so probit analysis could not be developed. Fecundity was reduced moderately at the lower doses and drastically at the higher doses. At doses causing 100% sterility no eggs were laid by the treated females. Egg viability was moderately reduced at the higher doses of the three day treatment but was only slightly affected at the two and one day treatments throughout the dose range.

As previously pointed out, the concentration of metepa or hempa needed for 100% sterility (infecundity) in the females was the same whether administered during the first one, two, or three days of adult life, namely 0.05% for metepa and 0.375% for hempa. Likewise the effects of other concentrations administered during two or three days were very similar to those obtained with a one day treatment.

The data indicate that in the female the treatments administered during the day of emergence were the most effective in causing infecundity. Similar results were reported by Crystal and LaChance (1963) and LaChance and Crystal (1963). These authors found that the effect of several alkylating agents as inhibitors of ovarian growth in the screw-worm fly was greatest when the treatments were applied to 0-4 hour old females, i.e. while the nurse cells were in the endomitotic phase. The same treatment given 24 hours later did not affect

the fecundity of the females. They concluded that apparently the endomitotic replication of the nurse cells was completely disrupted. Bertram (1964) also reported a similar effect on the female Aedes aegypti when treated with thiotepa.

The data also suggest that the stable fly is considerably more susceptible to the sterilizing action of metepa than the house fly when given in the food. The stable fly required a concentration of only 0.012% for three days, or 0.05% for one day to produce complete sterility in the male, and 0.05% for one, two, or three days to produce complete sterility in the female. Gouck et al. (1963) obtained 0% hatch in house flies when both sexes caged together were provided with 1% metepa in the sugar for the first three days after emergence thereafter feeding on fly food in addition to the treated sugar. Dame and Schmidt (1964) obtained high, but not complete sterility by allowing house flies to feed on 0.4% metepa in the fly food during the first three days after emergence. Parish and Arthur (1965) in turn administered metepa to house flies in a liquid diet composed of 1 part condensed milk and 1 part water. By providing freshly treated food every day during 14 days these authors obtained complete infecundity with a concentration of 0.5% metepa. They obtained very few eggs (all sterile) at concentrations of 0.25% and 0.125%.

When SC-50 values are compared, the stable fly is found to be about 10 times more susceptible to metepa than the house fly. For a three day treatment in the males, the SC-50 for the stable fly was 0.0020% and the SC-90 was 0.0051 (slope 3.17). The corresponding values for the house fly, as reported by Murvosh et al. (1964) were: SC-50, 0.022%; and SC-90, 0.121 (slope 1.73).

The feeding method used does not allow for a quantitative determination of activity (Chang et al., 1964) and therefore no determination of the dose per fly can be made from the data presented. However, other workers have tested metepa by injection or topical application in both the house fly and the stable fly.

In the stable fly, Harris (1962) obtained complete sterility when both sexes were treated with metepa, 3 micrograms per fly applied topically, and then mated with each other. This dosage did not induce infecundity in the females. Total infecundity resulted when the flies were treated with 10 micrograms per fly. In the house fly Chang and Borkovec (1964) obtained 99.3% sterility in the male house flies by injection of 8 micrograms of metepa per fly. The higher susceptibility of the stable fly to metepa holds, even considering that injection is more efficient than topical application, as is evident from Chang et al. (1964), who showed that the male house fly was completely sterilized with a dose of 40 micrograms of hempa per fly when applied by injection, or 200 micrograms per fly when applied topically.

The stable fly also appears to be somewhat more susceptible to hempa than the house fly. LaBrecque et al. (1966) did not obtain 100% sterility when male house flies were allowed to feed on 1% and 2.5% hempa in fly food for one, two, or three days. A five day treatment was necessary to obtain 100% sterility. When only an aqueous sugar solution containing 1% hempa was provided a one day treatment was sufficient to induce 100% sterility in the male. Complete sterility in the females was obtained after a three day treatment with 1% hempa in an aqueous sugar solution.

Chang et al. (1964) induced 100% sterility in male house flies by allowing them to feed on 0.25% hempa in a liquid fly food (50 parts

non-fat dry milk, 1 part sugar, and 49 parts water) for 24 hours. The results in the females were very erratic.

In the work herein reported both male and female stable flies were completely sterilized with 0.5% hempa in citrated blood for one day.

Although both metepa and hempa were effective chemosterilants of the stable fly, metepa was about 16 times more effective than hempa, as evidenced by the positions of the corresponding curves. The potency ratio between the three day curves was 15.75. The intensity of the action of the two chemosterilants, however, was very similar within their own range of activity, as can be inferred from the slopes of the lines, which did not differ significantly. The slope ratio between the two lines was 1.235 (Fig. 18).

Table 1.--Sexual sterility induced in stable flies treated orally with metepa in citrated blood during the first three days of adult life (summary of 3 replications)

Concentration %	Number of eggs	Number of pupae	Pupation %	Sterility % 1/
0.0250	585	0	0	100
0.0125	471	0	0	100
0.0062	564	24	4.25	95.17
0.0031	650	189	29.07	66.97
0.0015	613	324	52.85	39.96
0.0007	353	300	84.98	4.59
none	618	544	88.02	

1/ corrected by Abbott's formula

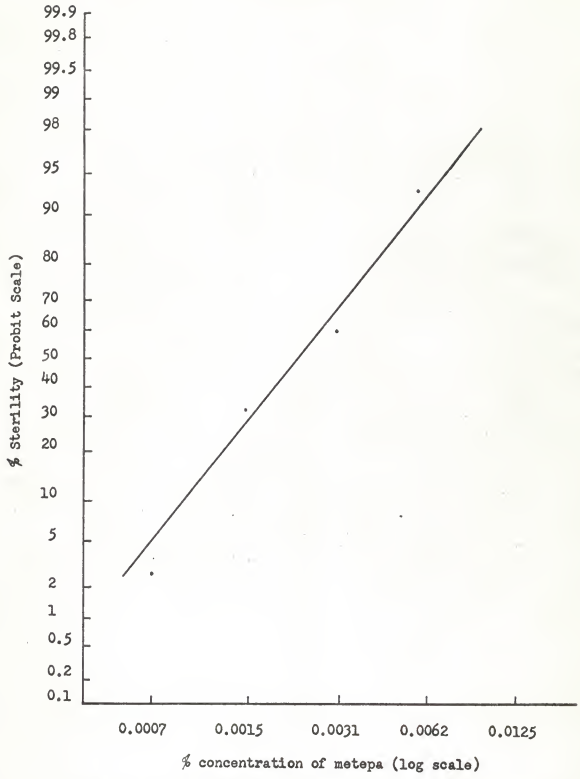

Fig. 10.--The dose-sterility curve for male stable flies treated orally with metepa in citrated blood during the first three days of adult life.

Table 2.--Sexual sterility induced in male stable flies treated orally with metepa in citrated blood during the first two days of adult life (summary of 3 replications)

Concentration %	Number of eggs	Number of pupae	Pupation %	Sterility % 1/
0.0500	595	0	0	100
0.0250	566	0	0	100
0.0125	623	32	5.13	94.17
0.0062	517	102	19.73	77.58
0.0031	484	220	45.45	48.36
0.0015	432	341	78.94	10.31
none	618	544	88.02	

1/ corrected by Abbott's formula

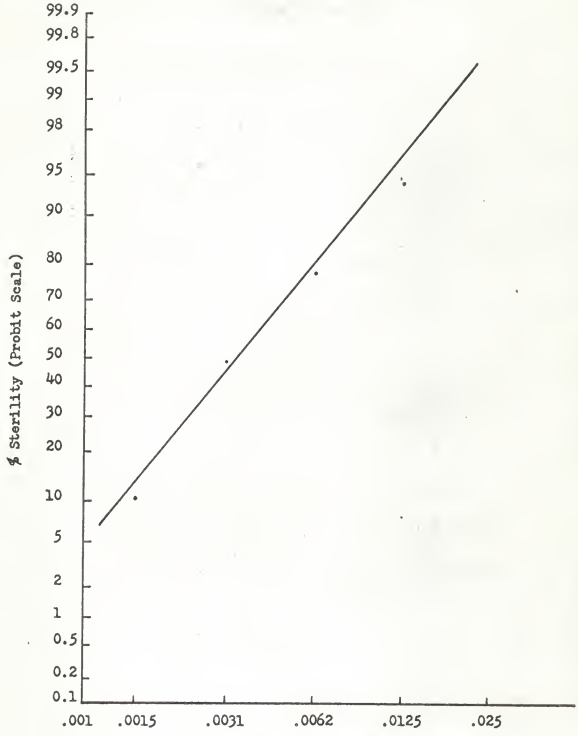

Fig. 11.--The dose-sterility curve for male stable flies treated orally with metepa in citrated blood during the first two days of adult life.

Table 3.--Sexual sterility induced in male stable flies treated orally with metepa in citrated blood during the first day of adult life (summary of 3 replications)

Concentration %	Number of eggs	Number of pupae	Pupation %	Sterility % 1/
0.0500	549	0	0	100
0.0250	605	4	0.66	99.26
0.0125	385	58	15.06	83.15
0.0062	430	99	23.02	74.25
0.0031	463	265	57.23	35.98
none	368	329	89.40	

1/ corrected by Abbott's formula

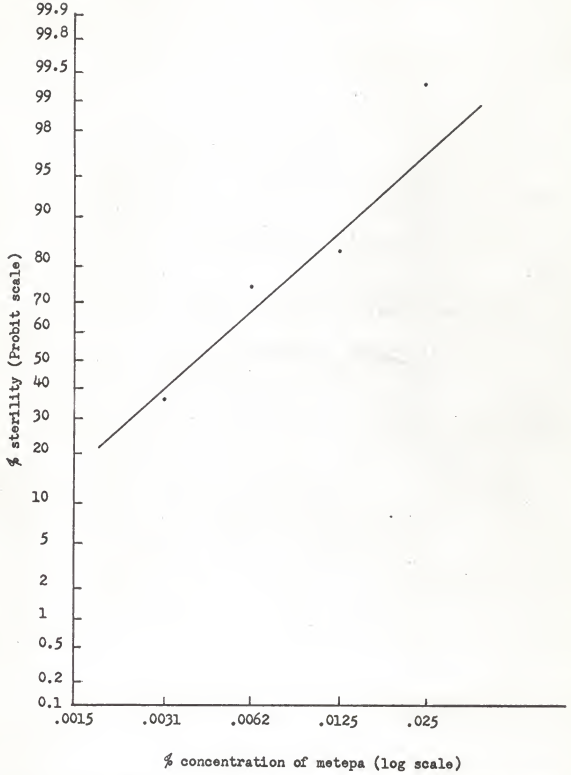

Fig. 12.--The dose-sterility curve for male stable flies treated orally with metepa in citrated blood during the first day of adult life.

Table 4.—Percent concentration of metepa in citrated blood required to induce three levels of sterility in the male stable fly in one, two, and three day treatments (95% confidence limits in parenthesis)

	Number of days under treatment		
	3	2	1
SC-50	0.0020	0.00335	0.0040
	(0.00156-0.00256)	(0.00259-0.00434)	(0.002 -0.0057)
SC-90	0.0051	0.00825	0.0145
	(0.0033-0.0078)	(0.0053-0.0126)	(0.0073-0.0288)
100%	0.0125	0.0250	0.050
Slope	3.17	3.18	2.28
Potency ratio	2.00	1.195	1.0

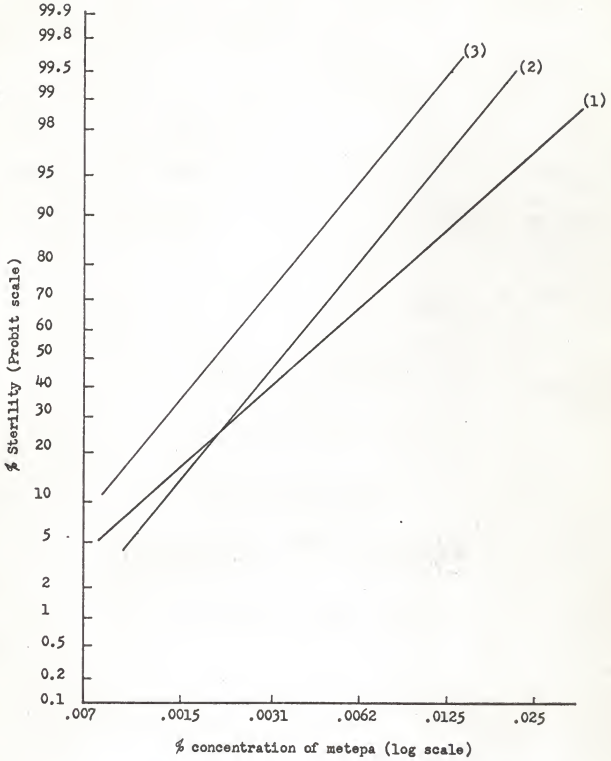

Fig. 13.--Comparison of the dose-sterility curves for male stable flies treated with metepa in citrated blood for one (1), two (2), and three (3) days.

Table 5.--Sexual sterility induced in male stable flies treated orally with hempa in citrated blood during the first three days of adult life (summary of 3 replications)

Concentration %	Number of eggs	Number of pupae	Pupation %	Sterility % 1/
0.250	515	0	0	100
0.125	470	0	0	100
0.062	429	44	10.26	89.00
0.031	421	180	42.76	52.66
0.015	280	237	84.64	6.30
none	300	271	90.33	

1/ corrected by Abbott's formula

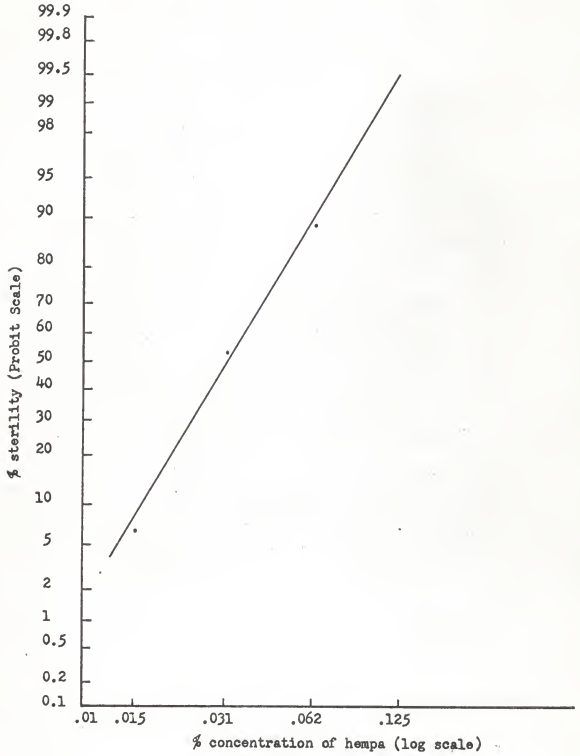

Fig. 14.--The dose-sterility curve in male stable flies treated orally with hempa in citrated blood during the first three days of adult life.

Table 6.--Sexual sterility induced in male stable flies treated orally with hempa in citrated blood during the first two days of adult life (summary of 3 replications)

Concentration %	Number of eggs	Number of pupae	Pupation %	Sterility % 1/
0.500	469	0	0	100
0.250	589	0	0	100
0.125	585	28	4.79	94.77
0.0625	607	161	26.52	71.09
0.0312	580	404	69.65	24.09
0.0151	553	398	71.97	21.67
none	595	546	9.76	

1/ corrected by Abbott's formula

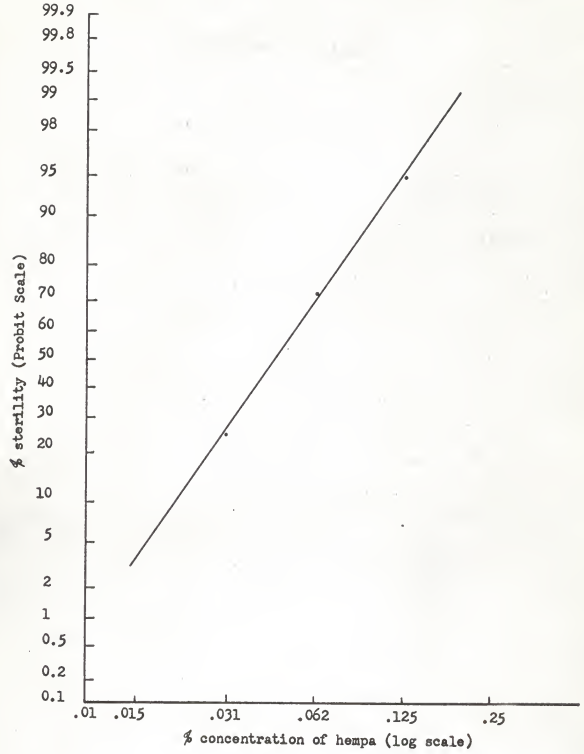

Fig. 15.--The dose-sterility curve for male stable flies treated orally with hempa in citrated blood during the first two days of adult life.

Table 7.--Sexual sterility induced in male stable flies treated orally with hempa in citrated blood during the first day of adult life (summary of 3 replications)

Concentration %	Number of eggs	Number of pupae	Pupation %	Sterility % 1/
0.500	484	0	0	100
0.250	300	8	2.66	96.95
0.125	167	47	28.14	67.77
0.0625	215	132	61.39	29.69
0.0312	211	171	81.20	7.00
none	291	254	87.31	

1/ corrected by Abbott's formula

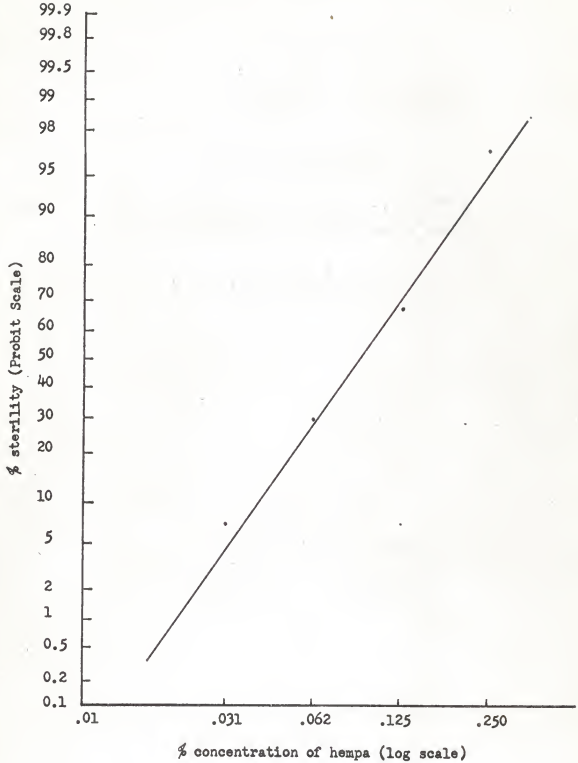

Fig. 16.--The dose-sterility curve for male stable flies treated orally with hempa in citrated blood during the first day of adult life.

Table 8.--The percent concentration of hempa in citrated blood required to induce three levels of sterility in the male stable fly in one, two, and three day treatments (95% confidence limits in parenthesis)

	Number of days under treatment		
	3	2	1
SC-50	0.0315	0.0455	0.090
	(0.024-0.0413)	(0.0368-0.0564)	(0.0720-0.113)
SC-90	0.0625	0.100	0.200
100%	0.125	0.250	0.500
Slope	3.91	3.73	3.68
Potency ratio	2.88	1.98	1.0

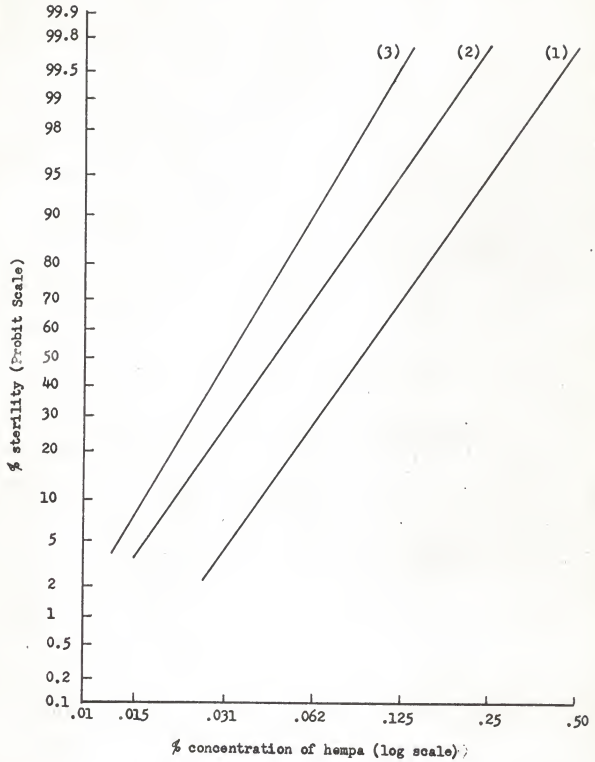

Fig. 17.--Comparison of the dose-sterility curves for male stable flies treated orally with hempa in citrated blood for one (1), two (2), and three (3) days.

Table 9.--Sexual sterility induced in female stable flies treated orally with metepa in citrated blood during the first three days of adult life (summary of 2 replications)

Concentration	Number of eggs	Number of eggs hatched	Fecundity %	Hatchability %	Sterility % 1/
0.0500	0	0	0	0	100
0.0375	32	22	2.60	69.37	98.20
0.0250	144	88	11.70	61.66	92.78
0.0187	209	135	17.00	65.18	88.92
0.0125	1,010	889	82.13	88.81	27.06
0.0062	1,200	1,101	97.60	92.58	9.64
0.0031	1,159	1,034	94.23	90.02	15.17
none	1,230	1,219	100	100	0

1/ % sterility 100 (1-fh)

Table 10.--Sexual sterility induced in female stable flies treated orally with metepa in citrated blood during the first two days of adult life (summary of 2 replications)

Concentration	Number of eggs	Number of eggs hatched	Fecundity %	Hatchability %	Sterility % 1/
0.0500	0	0	0	0	100.00
0.0375	70	50	3.61	73.44	97.34
0.0250	386	252	19.93	67.12	98.66
0.0187	888	757	45.84	87.64	59.82
0.0125	1,555	1,322	80.28	87.41	29.83
0.0062	1,856	1,717	95.82	95.11	8.87
0.0031	1,895	1,858	97.83	100.80	1.38
none	1,937	1,884	100.00	100.00	0

1/ % sterility: 100 (1-fh)

Table 11.--Sexual sterility induced in female stable flies treated orally with metepa in citrated blood during the first day of adult life (summary of 2 replications)

Concentration	Number of eggs	Number of eggs hatched	Fecundity %	Hatchability %	Sterility % 1/
0.0500	0	0	0	0	100
0.0375	157	67	8.21	46.84	96.15
0.0250	1,451	1,192	75.89	90.16	31.58
0.0187	999	765	52.25	84.05	56.09
0.0125	1,538	1,460	80.44	104.19	16.19
0.0062	1,784	1,708	93.30	105.30	1.75
0.0031	2,076	1,956	108.57	103.41	0
none	1,912	1,743	100.00	100.00	0

1/ % sterility: 100 (1-fh)

Table 12.--Sexual sterility induced in female stable flies treated orally with hempa in citrated blood during the first three days of adult life (summary of 2 replications)

Concentration	Number of eggs	Number of eggs hatched	Fecundity %	Hatchability %	Sterility % 1/
0.500	0	0	0	0	100
0.375	0	0	0	0	100
0.250	148	6	5.25	64.31	96.62
0.187	215	105	7.63	54.05	95.88
0.125	671	305	23.81	50.31	88.02
0.0625		917	41.95	85.86	63.98
0.0312	1,204	1,079	42.73	99.19	57.62
0.0156	925	886	32.83	106.00	34.80
none	2,818	2,546	100.00	100.00	0

1/ % sterility: 100 (1-fh)

Table 13.--Sexual sterility induced in female stable flies treated orally with hempa in citrated blood during the first two days of adult life (summary of 2 replications)

Concentration	Number of eggs	Number of eggs hatched	Fecundity %	Hatchability %	Sterility % 1/
0.500	0	0	0	0	100
0.375	117	40	5.48	3.44	97.89
0.250	263	106	12.32	45.31	94.42
0.187	502	307	23.52	68.75	83.83
0.125	953	787	44.66	92.84	58.54
0.0625	2,107	1,898	98.73	101.00	0
0.0312	1,316	1,161	61.67	99.17	38.84
0.0156	1,454	1,243	68.13	96.11	34.52
none	2,134	1,898	100.00	100.00	0

1/ % sterility: 100 (1-fh)

Table 14.--Sexual sterility induced in female stable flies treated orally with hempa in citrated blood during the first day of adult life (summary of 2 replications)

Concentration	Number of eggs	Number of eggs hatched	Fecundity %	Hatchability %	Sterility % 1/
0.500	0	0	0	0	100
0.375	0	0	0	0	100
0.250	756	513	46.35	68.99	68.02
0.187	636	446	38.99	71.30	72.22
0.125	1,229	1,063	75.35	87.95	33.73
0.0625	1,796	1,672	110	94.66	0
0.0312	1,642	1,353	100	83.78	15.66
0.0156	1,982	1,786	121	91.62	0
none	1,631	1,604	100.00	100.00	0

1/ % sterility: 100 (1-fh)

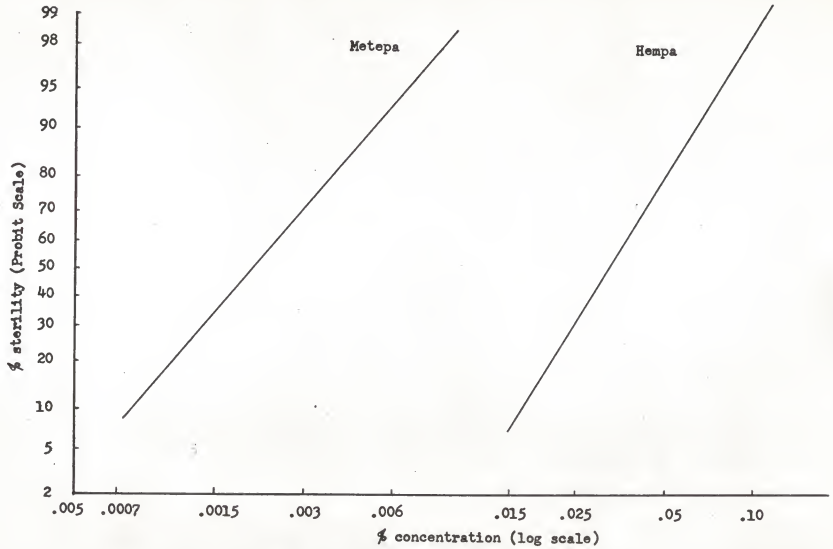

Fig. 18.--Comparison of the dose sterility curves for male stable flies treated orally with metepa or hempa in citrated blood during the first 3 days of adult life. (Potency ratio = 15.75)

Longevity Studies

The results of the longevity tests appear in tables 15 to 19 and the corresponding survivorship curves appear in Figs. 19 to 22. Such curves were constructed by plotting the percentage of surviving flies against the number of days elapsed.

By inspection of table 15 and Fig. 19 it can be seen that treatment with a male sterilizing dose of hempa for three days shortened the lifespan of virgin male stable flies, especially in the period immediately following the treatment and up to the 7th day. At this time only 65.6% of the hempa treated population survived, in comparison with 91.3% for a sterilizing dose of metepa and 90.6% for the control. The mean mortality occurs for males at 9 to 10 days. On the 9th day only 50.6% of the hempa treated population survived, compared to 63.3% for metepa and 68.1 for the control. However, there was no significant difference between the mean lifespan of the treated virgin males and the control (see table 19). The curves for metepa and the control followed virtually the same path, which suggest that metepa had little or no effect on the virgin male stable flies treated with a sterilizing dose. Essentially the same effects were obtained for non-virgin males, i.e. males caged with females at a 1:1 ratio from the time of emergence (see table 16 and Fig. 20).

The effect of the male sterilizing dose of metepa or hempa on the lifespan of virgin female stable flies is shown in table 17 and Fig. 21. It is apparent that both metepa and hempa reduce the lifespan approximately at the same rate. However, for the first 12 days hempa produced a greater mortality than metepa, as it did in the males. The mean lifespan of the treated virgin females was shorter by about

2.5 days than that of the controls (table 19). Such a difference was significant at the .05 level of probability.

The data obtained in tests with non-virgin females, i.e. females caged with males at a 1:1 ratio from the time of emergence, appear in table 18 and Fig. 22. Here also hempa displayed its early effect but on the 9th day the three curves tend to merge, so that the treatments apparently had no effect on the longevity of mated females. The mean lifespan of the treated non-virgin females did not differ significantly from that of the controls.

Under the conditions of this experiment it was observed that the females, both virgin and mated; treated or untreated, lived considerably longer than the males. Moreover, virgin females lived about 1 1/2 times as long as the mated females and twice as long was the virgin or non-virgin males. Virgin females lived an average of 19.53 to 22.21 days (range 3 to 38 days), while non-virgin females lived an average of 12.95 to 14.56 days (range 2 to 35 days). The mean lifespan of all males was 9.40 to 10.95 days (range 2 to 22 days).

One general conclusion to be drawn from the data here presented is that, except in the case of the virgin females, the mean lifespan of the stable fly was not reduced significantly be a three day treatment with a male sterilizing dose of metepa or hempa. Nevertheless a slight reduction of the lifespan was consistently observed in all treated fly populations when compared with the controls.

The early toxic effect displayed by hempa, however, is important inasmuch as the greatest sexual activity of the stable fly takes place in the earlier part of adult life. Harris et al. (1966) found that 89% of caged stable flies had mated by the 5th day. According to

LaBrecque (in press) it is during the early life that female house flies are first ready to accept mates and the mating competitiveness of the males is at its height.

Adverse effects on the longevity of insects treated with chemosterilants has been reported in the literature. Murvosh et. al. (1964) found that the longevity of house flies was reduced nearly 50% when apholate or metepa at both 0.5% and 1% was incorporated in the sugar part of the diet and offered to the flies during their lifespan. They observed, nevertheless, that more than 90% of the treated flies, males or females, were still alive on the 10th day, which was ample time for normal sexual activity to take place.

On the other hand, there is at least one report of increased longevity in insects following chemosterilization. LaChance et al. (in press) report a personal communication by I. Keiser to the effect that sterilization by tepa increased the longevity of male and female oriental fruit flies, Dacus dorsalis Hendel and female Mediterranean fruit flies, Ceratitis capitata (Wied.).

In the experiment reported here the reduced lifespan of both treated and untreated female stable flies caged with males since the day of emergence, in comparison with virgin females, is a result similar to that obtained by Baumhover (1965) with the screw-worm flies. He observed that female screw-worm flies, which are monogamous, experienced an increased mortality as a result of being harassed by aggressive males that attempted to mate repeatedly. This observation enabled Baumhover to devise a sexual aggressiveness test (SAG) based on the mortality of females caged with males at a ratio of 3♂ to 1♀.

In this respect it is also worthy of note that in the tests with the stable fly, there was no significant difference in longevity between treated and non-treated females caged with males (ratio 1:1) from the day of emergence (table 19). This might constitute further evidence that the mating competitiveness of the treated males is at least equal to that of normal males (See the results of mating competitiveness tests).

No reduction of longevity was observed in the males when caged with or without females since the day of emergence.

The length of life in insects is known to be affected by a complex of many factors, which include nutrition of the immature stages, nutrition of the adults, sex, sex ratio, ambient temperature, relative humidity, light intensity, photoperiod, population density, natural enemies, etc. Therefore the duration of the lifespan recorded here applies only to the conditions of this experiment and is not directly indicative of the lifespan of the stable fly in nature.

Table 15.--Percentage of survival of virgin male stable flies treated orally with sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life (summary of 3 replications)

Days	Metepa 0.0125%	Hempa 0.125%	Control
1	100	100	100
2	100	100	98.8
3	99.6	96.9	97.5
4	99.6	94.4	97.5
5	99.2	89.4	97.5
6	98.8	81.9	96.2
7	91.3	65.6	90.6
8	73.8	60.6	80.0
9	63.3	50.6	68.1
10	45.8	37.5	48.1
11	32.5	32.5	37.5
12	23.8	18.3	24.4
13	12.9	5.6	16.9
14	5.8	3.8	8.8
15	--	1.9	--
16	2.5	0.6	2.5
17	1.2	0.6	1.9
18	0.4	0	1.2
19	0.4		1.2
20	0		0

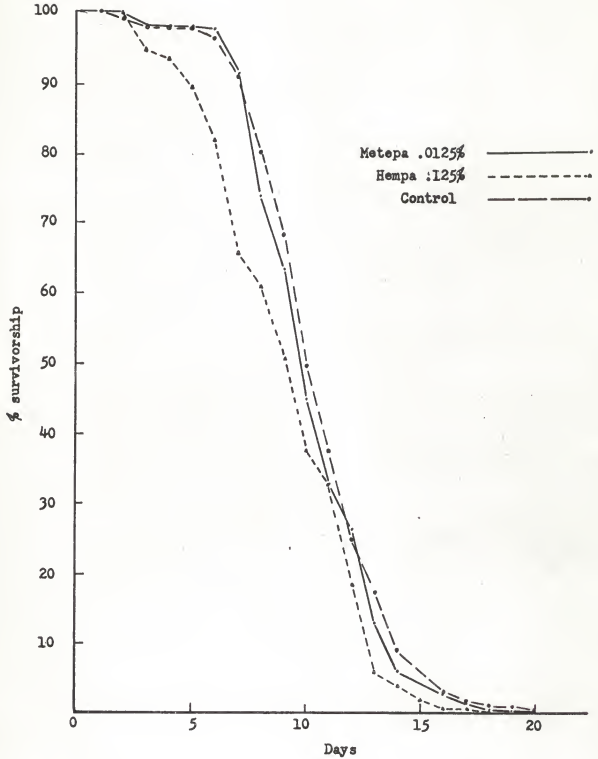

Fig. 19.--Survivorship curves of virgin male stable flies treated orally with sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life.

Table 16.--Percentage of survival of male stable flies caged together with females (ratio 1:1) and treated orally with sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life (summary of 3 replications)

Days	Metepa 0.0125%	Hempa 0.125%	Control
1	100	100	100
2	100	100	100
3	100	98.7	100
4	100	97.5	98.7
5	96.7	94.4	98.7
6	95.0	86.1	96.2
7	89.2	72.2	92.4
8	73.3	67.1	81.0
9	63.3	57.0	72.2
10	48.3	39.3	58.2
11	31.6	25.4	35.4
12	25.6	17.7	26.5
13	14.9	10.0	12.6
14	8.1	--	--
15	--	4.5	7.5
16	2.5	3.5	1.3
17	1.5	3.5	1.3
18	1.5	1.2	0
19	0	1.2	
20		1.2	
21		1.2	
22		0	

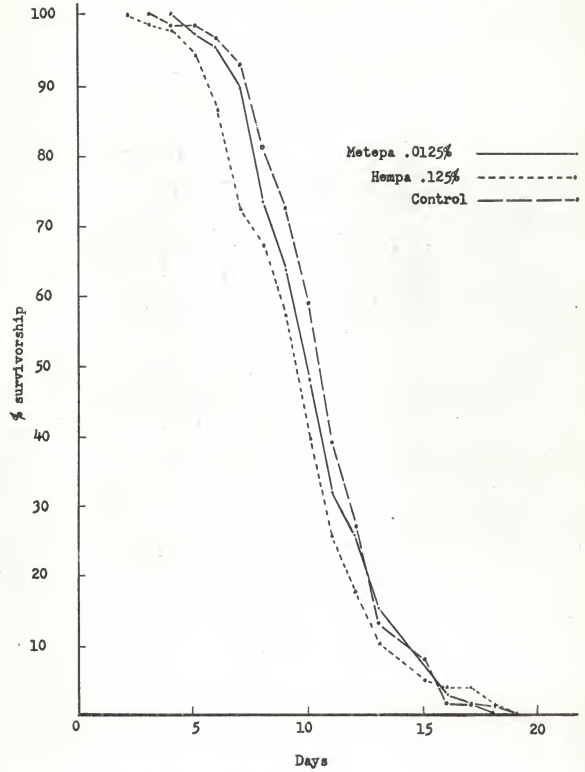

Fig. 20.--Survivorship curves for male stable flies caged together with females (ratio 1:1) and treated orally with sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life.

Table 17.--Percentage of survival of virgin female stable flies treated orally with male sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life (summary of 3 replications)

Days	Metepa 0.0125%	Hempa 0.125%	Control
1	100	100	100
2	100	100	100
3	100	96.8	100
4	100	96.8	98.7
5	98.8	96.8	98.7
6	97.9	95.5	97.1
7	97.1	93.6	96.8
8	95.4	90.4	96.8
9	94.6	87.3	95.5
10	93.1	84.7	94.3
11	88.3	83.4	90.1
12	81.2	80.3	89.8
13	75.3	75.8	87.3
14	70.7	71.3	82.8
15	--	66.9	--
16	61.1	63.1	72.6
17	58.6	57.9	68.2
18	53.1	53.5	63.1
19	49.8	49.0	60.5

Table.17--(Continued)

Days	Metepa 0.0125%	Hempa 0.125%	Control
20	43.1	45.2	55.4
21	38.5	40.1	50.3
22	33.9	31.8	46.5
23	28.4	29.3	40.1
24	27.6	24.2	39.5
25	23.0	21.0	33.8
26	19.7	19.1	28.0
27	15.9	15.9	23.6
28	13.4	14.8	21.0
29	11.7	12.7	19.1
30	10.0	11.5	17.8
31	7.9	11.5	15.9
32	7.9	9.6	14.6
33	5.9	8.3	13.4
34	4.2	--	10.8
35	--	3.8	--
36	1.7	--	1.9
37	1.7	0	1.9
38	0		0

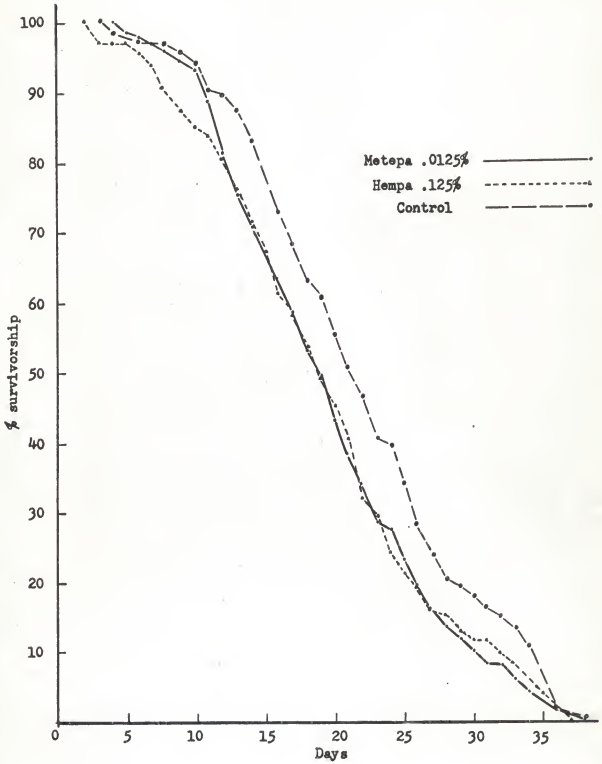

Fig. 21.--Survivorship of virgin female stable flies treated orally with male sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life.

Table 18.--Percentage of survival of female stable flies caged together with males (ratio 1:1) and treated orally with male sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life (summary of 3 replications)

Days	Metepa 0.0125%	Hempa 0.125%	Control
1	100	100	100
2	100	97.5	100
3	100	97.5	100
4	100	96.2	100
5	99.2	94.9	98.7
6	97.5	92.4	97.5
7	94.1	79.8	89.9
8	88.2	77.2	83.6
9	79.0	74.7	76.0
10	69.8	64.6	72.2
11	61.4	50.6	62.0
12	52.1	41.8	55.7
13	41.2	39.2	39.2
14	34.5	--	--
15	--	30.4	36.6
16	23.5	26.6	26.6
17	22.7	25.3	26.6
18	20.2	22.8	24.1

Table.18--(Continued)

Days	Metepa 0.0125%	Hempa 0.125%	Control
19	13.4	10.1	20.3
20	10.9	6.3	17.7
21	7.6	3.8	13.9
22	7.6	3.8	11.4
23	5.9	3.8	11.4
24	5.1	2.5	--
25	4.2	2.5	6.3
26	3.5	2.5	6.3
27	2.5	2.5	6.3
28	2.5	2.5	5.1
29	0.8	1.3	--
30	0.8	1.3	--
31	0.8	1.3	3.8
32	0.8	0	3.8
33	0.8		2.5
34	0.8		0
35	0.8		
36	0		

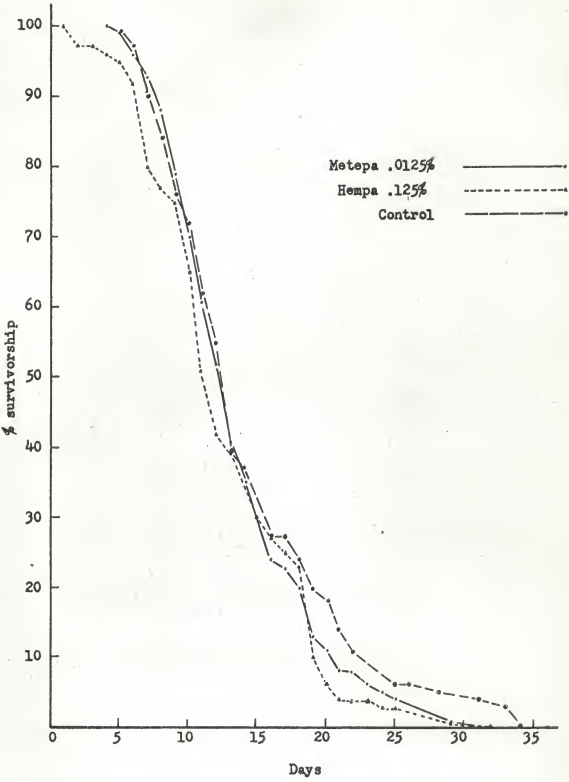

Fig. 22.--Survivorship curves for female stable flies caged together with males (ratio 1:1) and treated orally with male sterilizing doses of metepa and hempa in citrated blood during the first three days of adult life.

Table 19.--Mean lifespan of stable flies treated orally with male sterilizing doses of metepa and hempa in citrated blood during the first 3 days of adult life (Days)

	Metepa 0.0125%	Hempa 0.125%	Control
virgin males	10.69±0.24a	9.40±0.48a	10.77±0.46a
non-virgin males	10.61±0.52a	9.90±0.72a	10.95±0.60a
virgin females	19.98±0.91b	19.53±1.30b	22.21±1.26c
non-virgin females	13.86±0.99d	12.95±1.28d	14.56±1.50d

note: values followed by the same letter do not differ significantly

Mortality Studies

The results of the mortality tests appear in tables 20 to 23 and the corresponding dose-mortality curves appear in Figs. 23 to 26. The data indicate that both metepa and hempa are toxic to the stable fly over a narrow range of concentrations. However, it is not possible to make a direct comparison between the two chemosterilants because metepa was administered for a period of three days, while hempa was administered for one day only.

The characteristics of the dose-mortality curves appear in table 24. It is apparent that there was no significant difference between the susceptibility of the males or females to either metepa or hempa. In the case of metepa the LC-50 for males was 0.26% and that for females was 0.23%. For hempa the LC-50 in the males was 0.60% and in the females 0.63%.

Suitable comparisons were made between the curves for sterility and the curves for mortality for the two chemosterilants in male stable flies. These comparisons appear in Figs. 27 and 28. With these data it was possible to calculate safety factors (S.F.) as follows:

$$\text{metepa S.F.} = \frac{0.082 - 0.032}{0.032} = 1.56$$

$$\text{hempa S.F.} = \frac{0.21 - 0.90}{0.90} = -0.77$$

It can be seen that the safety factor for metepa is positive (S.F. : 1.56) indicating that it can produce 100% sterility in the male stable flies without causing any mortality. The data for hempa, on the other hand, yielded a negative safety factor (S.F. : -0.77) indicating that a high proportion of the population, 94% in this case, would

be expected to die before 100% sterility was attained with a one day treatment.

In practice, however, the safety margins were greater, as complete sterility in the males was actually obtained at lower concentrations than the values expected according to the dose-sterility curves. In a three-day treatment metepa caused complete sterility in the males at a concentration of 0.012% instead of 0.032% as expected, and hempa, in a one day treatment, produced complete sterility at 0.50% instead of 0.90% as expected. Nevertheless the safety factor for hempa would still be negative for a one day treatment.

On the basis of these results, it is apparent that metepa would be useful as a chemosterilant of the male stable fly, while hempa would offer little promise. This might well be the case, as in the longevity studies hempa also displayed an increased mortality during the first five days following a three day treatment with the male sterilizing dose.

Table 20.--Mortality induced in male stable flies treated orally with metepa in citrated blood during the first three days of adult life.

Concentration %	Number of flies	Number of dead flies	Mortality % 1/
0.750	100	100	100
0.500	100	100	100
0.375	100	90	90.0
0.250	100	40	39.4
0.187	100	19	18.2
0.125	100	0	0
none	100	1	0

1/ corrected by Abbott's formula

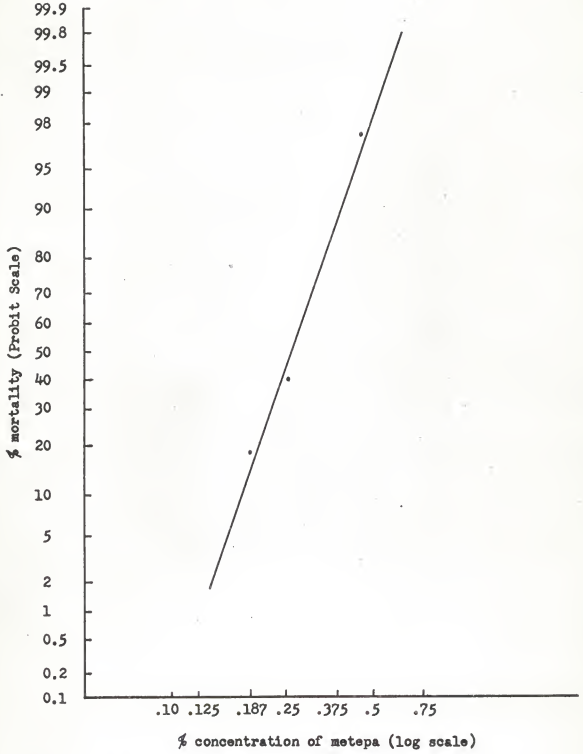

Fig. 23.--Dose-mortality curve for male stable flies treated orally with metepa in citrated blood during the first three days of adult life.

Table 21.--Mortality induced in female stable flies treated orally with metepa in citrated blood during the first three days of adult life.

Concentration %	Number of flies	Number of dead flies	Mortality % 1/
0.750	100	100	100
0.500	100	100	100
0.375	100	96	96.0
0.250	100	66	65.6
0.187	100	26	25.2
0.125	100	1	0
none	100	1	0

1/ corrected by Abbott's formula

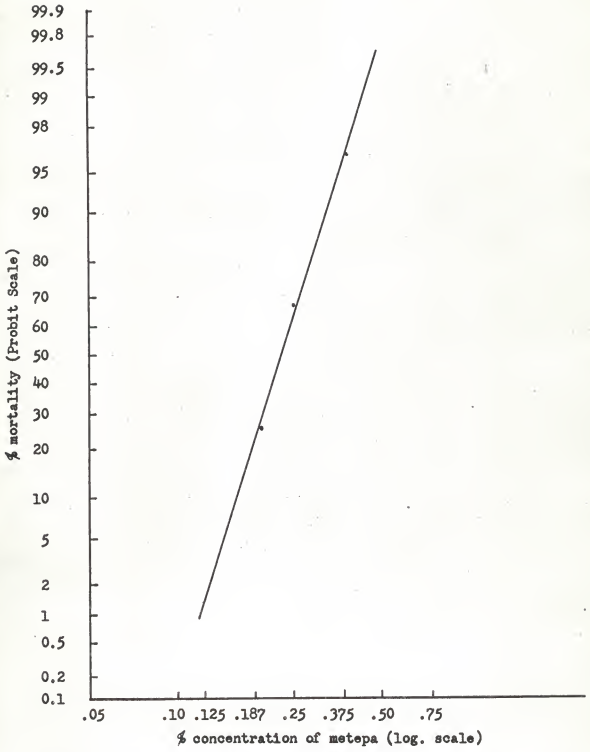

Fig. 24.--Dose-mortality curve for female stable flies treated orally with metepa in citrated blood during the first three days of adult life.

Table 22.--Mortality induced in male stable flies treated orally with hempa in citrated blood during the first day of adult life.

Concentration %	Number of flies	Number of dead flies	Mortality %
0.750	100	77	77.0
0.625	100	59	59.0
0.500	100	25	25.0
0.375	100	5	5.0
0.250	100	1	1.0
none	100	0	

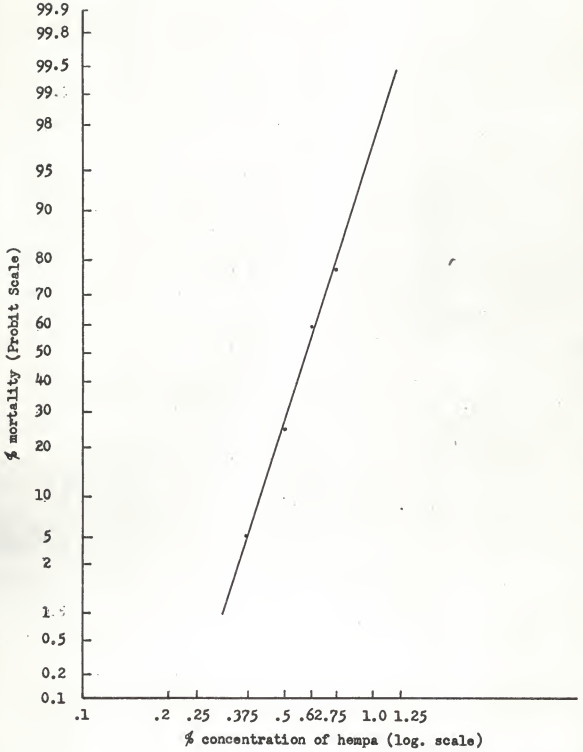

Fig. 25.--Dose-mortality curve for male stable flies treated orally with hempa in citrated blood during the first day of adult life.

Table 23.--Mortality induced in female stable flies treated orally with hempa in citrated blood during the first day of adult life.

Concentration %	Number of flies	Number of dead flies	Mortality %
0.750	100	73	73.0
0.625	100	48	48.0
0.500	100	20	20.0
0.375	100	0	0
0.250	100	0	0
none	100	0	0

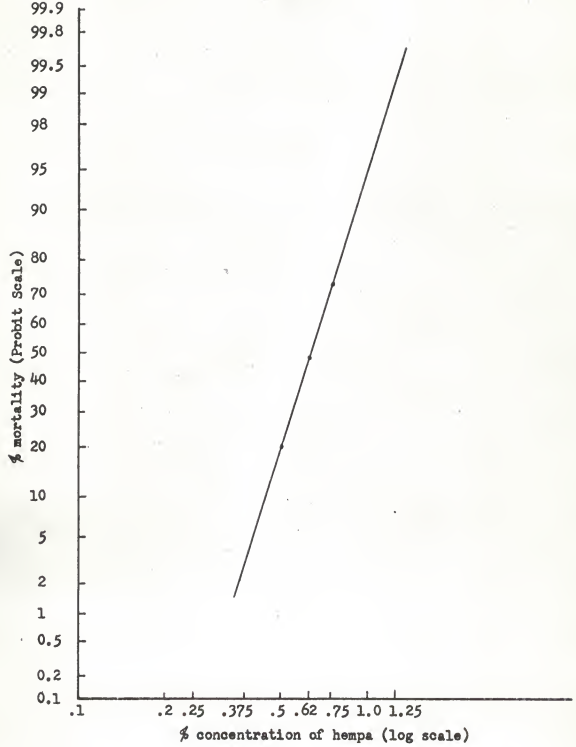

Fig. 26.--Dose-mortality curve for female stable flies treated orally with hempa in citrated blood during the first day of adult life.

Table 24.--Percent of metepa or hempa in citrated blood required to induce 2 levels of mortality in the stable fly when administered orally for the indicated period.

Sex of flies	Metepa		Hempa	
	First three days of adult life		First day of adult life	
	males	females	males	females
LC-50	0.260	0.23	0.60	0.63
	(0.24 - 0.28)	(0.22 - 0.24)	(0.57 - 0.63)	(0.60 - 0.66)
LC-90	0.38	0.33	0.85	0.90
	(0.36 - 0.41)	(0.31 - 0.34)	(0.81 - 0.90)	(0.84 - 0.96)
Slope	7.35	8.20	8.26	8.20

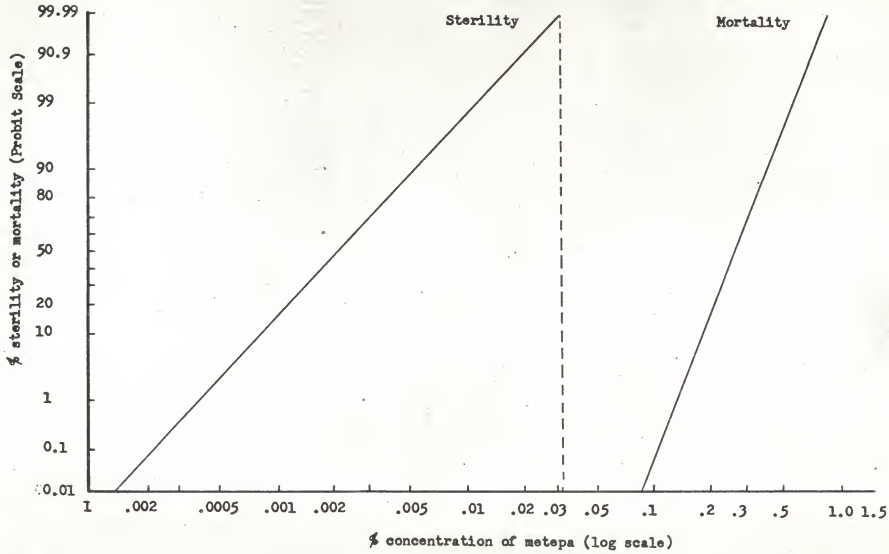

Fig. 27.--Comparison of dose-sterility and dose-mortality curves for male stable flies treated orally with metepa in citrated blood during the first three days of adult life.

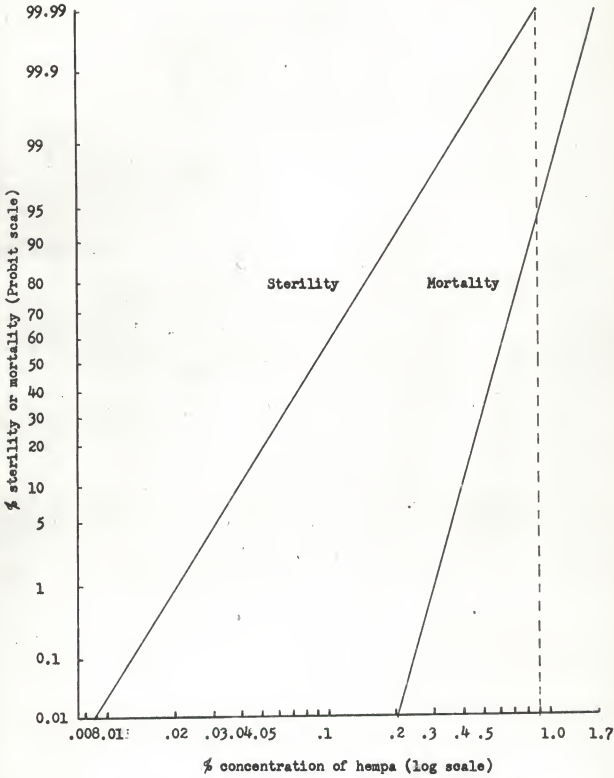

Fig. 28.--Comparison of dose-sterility and dose-mortality curves for male stable flies treated orally with hempa in citrated blood during the first day of adult life.

Studies on Mating Competitiveness

Mating competitiveness of sterile males is one of the requisites of the sterility method of population control (Knipling, 1964) and therefore it becomes necessary to evaluate the effect of sterilizing agents on this aspect of the biology of each species concerned.

The results of the tests on mating competitiveness appear in table 25. The data show that the percent sterility observed at different ratios of treated to normal males was either considerably greater (series I) or only slightly lower (series II) than the expected values. Such results indicate that male stable flies sterilized with metepa may be as successful or perhaps more successful than normal males in competition for mates.

These results are similar to those obtained by LaBrecque et al. (1962) in male house flies sterilized by feeding on fly food which contained 1% apholate. The percent of sterile eggs recorded by these authors was consistently greater than the expected values, which suggested a possible potentiation of the sterilized males in comparison with the normal males. Another explanation advanced by the authors was that mating by sterile males might in some way nullify the effects of prior matings by normal males.

Presently there is evidence which points to potentiation in chemosterilized male house flies. Kung (1967) placed groups of 10 treated or untreated male house flies in separate cages and then allowed them to mate with an equal number of untreated females. He found that within one minute four treated males were already mating as compared with only one in the untreated group.

The effect of sterilizing agents on the mating competitiveness of male insects seems to be rather specific. Davis et al. (1959) reported data which indicated that irradiation of pupae of Anopheles quadrimaculatus with 11,820 r of gamma radiation gave rise to males whose mating competitiveness was lower than that of normal males. Dame and Schmidt (1964) noted poor insemination due to reduced male vigor in mosquitoes (Anopheles quadrimaculatus and Aedes aegypti) exposed to a film of metepa 10 mg/sq ft. on glass surfaces for four hours.

On the other hand there are many reports on chemosterilants which do not reduce the mating competitiveness of the males. LaBrecque et al. (1962) reported increased mating competitiveness in male house flies treated with apholate, and Crystal (1965) has reported the finding of the first 3 chemosterilants which did not impair mating competitiveness of male screw-worm flies, as had all the effective chemosterilants tested before in the same insect. These were bis(1-aziridinyl) (hexahydro-1 H-azepin-1-yl)phopsphine oxide; 1- bis(1-aziridinyl)phosphinyl -3-(3,4-dichlorophenyl)-urea and N,N' tetramethylenebis (1-aziridinecarboxamide). Males treated with the latter even surpassed normal or radiosterilized males in mating competitiveness by a factor of about 4.

Clearly this enhancing of mating competitiveness induced by some chemosterilants in certain insects represents a distinct advantage which gives the chemosterilant approach a greater promise in population control.

Table 25.--Sterility obtained when normal female stable flies were caged with normal and/or metepa sterilized males at various ratios.

TM: NM: NF	Number of females	Number of eggs	Percent sterility		
			Observed	Corrected 1/	Expected
		Series I			
1 : 1 : 1	33	3,659	63.84	60.19	50.00
1 : 2 : 1	25	2,760	59.24	55.13	33.33
2 : 1 : 1	25	2,077	71.26	68.36	66.67
1 : 0 : 1	50	6,710	100.00	100.00	100.00
0 : 1 : 1	50	4,834	9.16	0	0
		Series II			
1 : 1 : 1	33	9,759	52.44	48.12	50.00
1 : 2 : 1	25	8,603	34.78	28.86	33.33
2 : 1 : 1	25	5,280	65.98	62.89	66.67
1 : 0 : 1	50	5,311	100.00	100.00	100.00
0 : 1 : 1	50	3,728	8.32	0	0

(TM: treated males; NM: normal males; NF: normal females. Summary of 3 replications per series).

SUMMARY

Several series of experiments were conducted to determine the effect of metepa (tris(2-methyl-1-aziridinyl)phosphine oxide) and hema (triz(dimethylamino)phosphine oxide) as chemosterilants of the stable fly, Stomoxys calcitrans (Linn).

Metepa induced complete sterility in male stable flies when provided in citrated blood to newly emerged adults at concentrations of 0.0125% for three days, 0.0250% for two days, or 0.05% for one day. The concentration for complete sterility in the females was 0.05% for either one, two, or three days.

Hempa induced complete sterility in the males when provided in citrated blood to newly emerged adults at concentrations of 0.125% for three days, 0.250% for two days, or 0.50% for one day. The concentration for complete sterility in the females was 0.375% for either one, two, or three days.

Normal females mated to the chemosterilized male produced a normal number of eggs. Hatchability was not established. Nevertheless no progeny developed to the pupal stage. The chemosterilized females produced no eggs.

A three day treatment with a male sterilizing dose of metepa or hempa significantly reduced the mean lifespan of virgin female stable flies. The same treatment did not cause a significant reduction in the mean lifespan of non-virgin females or males. Hempa, however, displayed an early toxic effect immediately after the treatment period in both males and females.

Virgin females lived about 1 1/2 times as long as non-virgin females and twice as long as virgin or non-virgin males. Virgin females lived an average of 19.53 to 22.21 days (range 3 to 38 days), while non-virgin females lived an average of 12.95 to 14.56 days (range 2 to 35 days). The mean lifespan of all males was 9.40 to 10.95 days (range 2 to 22 days).

Both metepa and hempa were toxic to the stable fly over a narrow range of concentration. A three day oral treatment with metepa in citrated blood caused mortality over a range of concentrations from 0.082% to 0.8%. A one day oral treatment with hempa in citrated blood caused mortality over a range of concentrations from 0.21% to 1.7%.

Male stable flies sterilized orally by a three day treatment with metepa at 0.0125% in citrated blood were as successful or perhaps more successful than normal males in competition for mates. Normal females confined with sterile and normal males at different ratios laid sterile eggs in a proportion either considerably higher or only slightly lower than expected.

Metepa appears to be a promising chemosterilant of the stable fly. Its safety ratio was 1.56, which means that there was ample margin between the minimum male sterilizing dose and the maximum tolerated dose. On the other hand hempa appears to be less promising than metepa as a chemosterilant of the stable fly. Its saftey ratio was -0.77, which, when related to the dosage-sterility and dosage-mortality curves, indicated that a high proportion of the male population would die before attaining complete sterility.

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BIOGRAPHICAL SKETCH

Jose de Jesus Castro Umana was born August 10, 1924, in Morales, Departamento de Izabal, Guatemala, Central America. He received his primary and secondary education in Guatemala City and in May, 1944, received the degree of Perito Agronomo from the National School of Agriculture in Guatemala.

In September, 1947, Mr. Castro entered Iowa State College, Ames, Iowa, U.S.A., and received the degree of Master of Science, major in Entomology in December, 1951. He has served the Guatemalan government in the capacity of Research Entomologist and ultimately as leader of coffee culture research. From July, 1955, to August, 1957, Mr. Castro served as the first Executive Director of the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA, International Regional Organization for Plant and Animal Protection) comprising Mexico, Central America, and Panama. Since January, 1962, he has been Professor of Entomology in the Facultad the Agronomia, Universidad de San Carlos, Guatemala. He has been on leave of absence beginning September, 1964, when he entered the University of Florida to work toward the degree of Doctor of Philosophy under a scholarship from the Rockefeller Foundation, graduating August, 1967.

Jose de Jesus Castro Umana is married to the former Morelia del Carmen Arriola Mencos and is the father of three children. He is a member of the Entomological Society of America; the Honor Society of Agriculture, Gamma Sigma Delta; The Society of the Sigma Xi; and the Colegio de Ingenieros y Arquitectos of Guatemala.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dean, College of Agriculture

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Supervisory Committee:

Chairman